केन्द्रीय समुद्री मात्स्यिकी अनुसंधान संस्थान
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
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P. B. No. 1603, Tatapuram, P. O, COCHIN-682014
केरल, भारत/KERALA, INDIA

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STUDIES ON THE REPRODUCTIVE BIOLOGY, BREEDING AND LARVAL REARING OF SELECTED MARINE ORNAMENTAL FISHES BELONGING TO THE FAMILY POMACENTRIDAE

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FOR
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OF THE
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To

My beloved parents

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CERTIFICATE

Certified that the thesis entitled " **STUDIES ON THE REPRODUCTIVE BIOLOGY, BREEDING AND LARVAL REARING OF SELECTED MARINE ORNAMENTAL FISHES BELONGING TO THE FAMILY POMACENTRIDAE** " is a record of independent research work carried out by **Mr. Sreeraj, G.** during the period of study from September 1998 to June 2002 under our supervision and guidance for the degree of **Doctor of Philosophy** in **Fish and Fisheries science (Mariculture)** and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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I hereby declare that the thesis entitled **"STUDIES ON THE REPRODUCTIVE BIOLOGY, BREEDING AND LARVAL REARING OF SELECTED MARINE ORNAMENTAL FISHES BELONGING TO THE FAMILY POMACENTRIDAE."** is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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SREERAJ, G.

सारांश

समुद्री जलजीवशालाओं के लिए अनुयोज्य मछलियाँ होने वाले सबसे प्रमुख कुटुम्बों में एक है पोमासेन्ट्रिडे कुडुम्ब. स्फुटनशाला में इनके उत्पादन की प्रौद्योगिकियाँ विकसित करके प्राकृतिक आवासों से इनका अति विदोहन रोकने हेतु प्रग्रहण प्रजनन और डिंभकों के पालन पर ध्यान देना आवश्यक है. इस अध्ययन में भारत के दक्षिण पश्चिम तट पर पाए जाने वाले साधारण पोमासेन्ट्रिडों का वर्गीकरण ; दो जातियों के लिंग विपर्यय पर ज़ोर देते हुए पुनरुत्पादी जीव विज्ञान; सात जातियों के ब्रूडस्टॉक विकास, प्रग्रहण प्रजनन तथा भ्रूणविज्ञान ; पांच जातियों के डिंभक पालन और कायांतरण पर अनुसंधान किया गया है. इस अध्ययन केलिए 8 वंश की 24 जातियों का संग्रहण किया गया जिनमें छः जातियाँ इस क्षेत्र में पहली बार रिकार्ड की गई हैं. *एम्फीप्रियोन सीबे* तथा *नियोपोमासेन्ट्रस सयानोमस* नामक दो जातियों के पुनरुत्पादी जीव विज्ञान पर अनुसंधान किया गया. इनमें *ए.सीबे* पुंपूर्वी उभयलिंगी और *एन. सयानोमस* स्त्रीपूर्वी उभयलिंगी है. *ए. सीबे* के नर जनन ग्रंथि एक अंडपृषण था जिसमें शुक्र जननीय सिस्ट तथा अपरिपक्व अंडक मौजूद थे. *एन.सयानोमस* में उभयलिंगी जननग्रंथि नहीं थी. ए. सीबे और *एन. सयानोमस* के किशोरों में ब्रूडस्टॉक का विकास किया गया. *ए. सीबे* के ब्रूड स्टॉक टैंक में एकसंगामनी युग्म का विकास किया गया जो उसी टैंक के अन्य मछलियों की अपेक्षा बडा था. *एन. सयानामस* का प्रजनन करने पर केवल एक प्रकार्यात्मक नर और बाकी सब स्त्री जाति थी. ए. सीबे की प्रग्रहण अवस्था में, प्रकार्यात्मक मादा मछली को निकालकर एक उप वयस्क (सबअडलट) को जोडने पर प्रकार्यात्मक नर में नर से मादा तक का रूपांतरण देखा गया. 61-135 दिनांक की अवधि में रूपांतरण हो जाता है. *एम्फीप्रियोन सीबे, नियोपोमासेन्ट्रम सयानोमस, एन.नीमुरस , एन.सिन्डोन्सिस, पोमासेन्ट्रस* सीरुलस, *पी. पावो* और *डासिलस केमियस* में प्रग्रहण प्रजनन किया गया. अध्ययन की गई सभी जातियाँ, लगातार प्रजनन करने वाली थी और अंडजनन में चांद्र आवर्तिता का प्रभाव नहीं पडा था. अंड धरातल पर चिपके जाते हैं और मुख्यतः नर जाति अंडों की सुरक्षा करते हैं. सभी जातियों में सूर्यास्त के बाद अंडों का स्फुटन हो जाता है और डिंभक वेलापवर्ती होते हैं. पांच जातियों में डिंभकों का पालन किया गया. औसत अतिजीविता दर 35.4% देखी गई और ऐनमोन मछली में अतिजीविता दर का रेंच 6.6% से 74% था. चार जातियों के कायांतरण पर भी अध्ययन किया गया. इस अध्ययन के परिणाम ऐनिमोन मछलियों और डामसेल मछलियों के ब्रूडस्टॉक विकास, प्रजनन और डिंभक पालन के मूल भूत पहलूओं पर प्रकाश डालते हैं और इन परिणामों को इन मछलियों के स्फुटनशाला उत्पादन की प्रौद्योगिकियों के विकास केलिए उपयुक्त किया जा सकता है.

ABSTRACT

The family Pomacentridae constitutes one of the most important families of fishes suitable for marine aquaria. Research on their captive breeding and larval rearing deserves priority attention to develop hatchery production technologies, which in turn can prevent their overexploitation from natural habitats. In the present study systematics of the common pomacentrids available along the South West coast of India, reproductive biology with emphasis on sex reversal of two species, broodstock development and captive breeding of seven species and their embryology, larval rearing and metamorphosis of five species were carried out. A total of 24 species belonging to 9 genera were collected in the present study of which six species were new records from the region. Reproductive biology of two species namely *Amphiprion sebae* Bleeker 1856 and *Neopomacentrus cyanomos* (Bleeker) 1856 were investigated. *Amphiprion sebae* was a protandrous hermaphrodite and *Neopomacentrus cyanomos* was a protogynous hermaphrodite. Male gonad of *Amphiprion sebae* was an ovotestis containing spermatogenic cysts and immature oocytes. Ambisexual gonads were not available from *Neopomacentrus cyanomos*. Broodstock development from juveniles was done for *Amphiprion sebae* and *Neopomacentrus cyanomos*. Monogamous pair was developed in each broostock tank of *Amphiprion sebae*, which were conspicuously larger than other members in the same tank. In *Neopomacentrus cyanomos* only one functional male developed in a breeding group and all others were females. Transformation from male to female occurred in functional male of *Amphiprion sebae* in captivity when the functional female was removed and a subadult was introduced. The transformation period ranged from 61 – 135 days. Captive breeding was done for *Amphiprion sebae, Neopomacentrus cyanomos, Neopomacentrus nemurus* (Bleeker) 1857, *Neopomacentrus sindensis* (Day) 1873, *Pomacentrus caeruleus* Quoy and Gaimard 1835, *Pomacentrus pavo (Bloch) 1787* and *Dascyllus carneus* Fischer 1885. All the species studied were continuous breeders and did not show any lunar periodicity of spawning. Eggs were attached to the substratum and the nest guarding was mostly done by the males. The eggs hatched after sunset and the larvae were pelagic for all the species. Larval rearing was done for five species. The average survival rate was 35.4 % and ranged from 6.6 % to 74 % in the anemonefish. The metamorphoses of four species were also studied. The results of the present study indicate the basic aspects of broodstock development, breeding and larval rearing of anemonefishes and damselfishes, which can be, utilized in the development of hatchery production techniques of these fishes.

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1. INTRODUCTION

Aquarium keeping is a popular hobby the world over and is second only to photography (Alava and Gomes, 1989). Man's curiosity towards aquatic environment and underwater life had led to the development of this hobby. A miniature form of aquatic ecosystem is created in an aquarium and hence this hobby, a challenging avocation, requires scientific knowledge, constant attention and innovations. The attraction and species diversity of marine ornamental fishes had always fascinated the aquarists, which inspired them to take up marine aquarium keeping. Eventhough many marine fishes suitable for aquaria were reported and described in the nineteenth century itself, marine aquarium keeping did not gain popularity among aquarists even in the first half of the twentieth century. The main reason for this was the scarcity of information on ecological, behavioural and physiological aspects of these fishes and the lack of technical knowledge on marine aquarium keeping. However, recent studies made extensively on the wild populations of reef fishes had yielded a wealth of scientific knowledge on their ecology, feeding habits, reproduction and social behaviour. This, coupled with the advent of sophisticated aquarium gadgets, enabled the aquarists to maintain marine aquaria successfully. The growth of marine aquarium hobby has led to an increased demand for marine ornamental fishes and consequently a lucrative marine ornamental fish trade has emerged on a global basis.

Maintaining a marine aquarium requires a different type of scientific and technological approach from that for a freshwater one. The higher pH of seawater is one of the major factors contributing to the complexity in the maintenance of a marine aquarium. While the pH of unpolluted freshwater is around 7.0, it ranges from 8 to 8.2 for unpolluted seawater. The toxicity due to unionised ammonia will be very high for seawater due to its alkaline nature when compared to neutral freshwater. Hence, ammonia detoxifying devices are

obligatory in a saltwater aquarium. Biological filters and carbon filters can significantly reduce the level of ammonia formed in the aquarium. In addition, a wide array of aquarium gadgets are now available in the market for monitoring and maintaining the water quality.

The desired qualities of an aquarium animal are mainly its attractiveness, hardiness, ability to accept artificial feeds, resistance to diseases and parasites, easy availability and ease of propagation. A variety of reef fishes have many of these qualities. Anemonefishes and damselfishes (Family : Pomacentridae), butterflyfishes and bannerfishes (Family : Chaetodontidae), angelfishes (Family : Pomacanthidae), cardinal fishes (Family : Apogonidae), wrasses (Family : Labridae) and parrot fishes (Family : Scaridae) are the major groups of fishes widely maintained in marine aquaria.

Family Pomacentridae includes about 320 species which occur in coral reef areas and shallow rocky seas (Allen, 1991). Their small size, bright colours and attractive behavioural patterns make them popular to marine aquarists. Most of the damselfishes are territorial and resident forms having restricted movements around the coral colonies where they are settled. They are mostly omnivorous, feeding on algae, zooplankters and a wide variety of other invertebrates (Allen, 1991). This diversity in feeding habit makes them to adapt quickly to the feeds provided in captive environment. Moreover, many pomacentrids attain sexual maturity and breed in captivity. They attach the eggs on submerged objects and exhibit parental care, which are added advantages for their hatchery production.

Pomacentrids are wildly distributed in the coral seas and are often associated with branching coral heads, rocky crevices or among the tentacles of sea anemones. The habitat preferences of many species of pomacentrids have been described in the early records on this group (Myrberg *et al.*, 1967; Swerdloff, 1970; Mariscal, 1970a, 1970b ; 1972 ; Clarke, 1971). The association

of anemonefishes and sea anemones has been a subject of interest for marine biologists for over a century. Various aspects of this association, *viz.* benefits to the host and fish, mechanisms of host location, specificity and biochemical aspects have been subjected to extensive studies. The mechanism of protection from sea anemones was subject to intensive studies and various theories were suggested (Schlichter, 1972; Elliott *et al.*, 1994; 1995; Mebs, 1994; Elliott and Mariscal, 1996).

Eventhough a wealth of information on the breeding and larval development is available from studies on the wild populations, captive breeding and rearing techniques of most of the species have not yet been perfected for commercial level production. Only twelve species of anemone fishes have been reared in captivity in large numbers (Arvedlund *et al.*, 2000a). The larval rearing of *Dascyllus aruanus* and *D. albisella* (Danilowicz and Brown, 1992), *Pomacentrus amboinensis, P. coelestis* and *Neopomacentrus bankieri* (Job *et al*, 1997), *Abudefduf saxatilis* (Alshuth *et al.*, 1998) and *Microspathodon chrysurus* (Pothoff *et al.*, 1987) have been reported. But all these were trials done on experimental levels, and large scale production methods are yet to be developed.

The present marine ornamental fish trade the world over is entirely dependant on natural stocks. The increasing demand for marine ornamental fishes has resulted in overexploitation of their wild stock and consequent destruction of reef areas (Alava and Gomes, 1989). The reefs are the results of hundreds of years of reef building activity, and the biodiversity of these delicate ecosystems has to be protected for posterity. The indiscriminate exploitation of reefs may convert the coral paradises into the graveyards of reef animals. In this context, the captive breeding and hatchery production of marine ornamental fishes assumes great significance. Hatchery produced fishes will prove to be an eco-friendly approach towards the development of a global marine ornamental fish trade. Information on the reproductive biology, breeding patterns and larval

rearing methods are the major prerequisites for their production in hatcheries, and hence intensive studies on these aspects are required to achieve the goal. In India, research on marine ornamental fishes is still in its infancy. Comprehensive information on the taxonomy, distribution and ecology is scanty for many groups of marine ornamental fishes including pomacentrids from Indian waters. Investigations on the ecological and reproductive aspects of reef fishes have to be undertaken from the wild habitat. Research on captive broodstock development, breeding and larval rearing has to be initiated.

In India, pomacentrids are widely distributed in the island ecosystems of Lakshadweep and Andaman – Nicobar group and mainland reef areas such as Gulf of Kutch, Gulf of Mannar and Palk Bay and thus offering potential to develop a trade for many species of pomacentrids. With this in view, a research programme on pomacentrid fishes with emphasis on their reproductive biology and breeding was undertaken, to acquire valuable information that can be applied for the hatchery production of these fishes. The major objectives of the present study are the following :

i. To study the taxonomy and distribution of commonly available pomacentrids.

ii. To gather sufficient information on their reproductive biology required for captive breeding.

iii. To study the behavioural aspects associated with breeding.

iv. To investigate in detail the early embryonic development and larval development.

v. To develop methods for their captive breeding and larval rearing.

In the present investigation, a systematic account of commonly available pomacentrids in Indian water especially along the southwest coast of India is given in Chapter 3. Description of the important reproductive biological

aspects related to sex reversal and mating system of two species of pomacentrids *viz. Amphiprion sebae* Bleeker 1853 and *Neopomacentrus cyanomos* (Bleeker) 1856 is given in Chapter 4. A detailed account of the broodstock development from juvenile populations of *Amphiprion sebae* and *Neopomacentrus cyanomos*, and captive breeding patterns and breeding behaviour of seven species *viz. Amphiprion sebae*, *Neopomacentrus cyanomos*, *Neopomacentrus nemurus* (Bleeker) 1857, *Neopomacentrus sindensis* (Day) 1873, *Pomacentrus caeruleus* Quoy and Gaimard 1835, *Pomacentrus pavo* (Bloch) 1787 and *Dascyllus carneus* Fischer 1885 is given in Chapter 5 and their embryology in Chapter 6. Chapter 7 includes the larval rearing methods for the clownfish and four damselfishes and larval development of four species of pomacentrids namely *Amphiprion sebae*, *Neopomacentrus cyanomos*, *Pomacentrus caeruleus* and *Pomacentrus .pavo*.

2. REVIEW OF LITERATURE

2.1. Introduction

The family Pomacentridae which is one of the largest families of reef fishes includes about 320 known species of fishes which are distributed in the coral reef areas and shallow rocky seas. A vast majority of these species have been reported from the tropical Indo- West Pacific region (Allen, 1991). There are various reports on the occurrence of pomacentrid fishes from Indian waters also. Jones and Kumaran (1980) described 35 species of pomacentrid species from the Laccadive archipelago. Kuthalingam *et al.* (1979) reported two species of pomacentrids from Indian waters. Pillai *et al.* (1992) recorded the commonly occurring fishes of Lakshadweep including pomacentrids. Vijayanand and Varghese (1990) reported that labrids and pomacentrids are the most commonly distributed fishes in Lakshadweep. Four species of pomacentrids were collected and their population characteristics were studied by Gopakumar *et al.* (1991). Allen (1991) recorded 41 species of pomacentrids from Indian waters. Murty (2002) reported 25 pomacentrids from Lakshadweep.

2.2. Ecology

Pomacentrids are generally resident fishes which do not move long distances from their territory (Allen, 1991). They are territorial species and establish their individual areas on dead coral rubbles. Different species occupy the same coral head but no species has a constantly greater efficiency for settlement than others and hence chance events may play an important role in determining their distribution (Sale, 1978). Many pomacentrids, especially

anemonefishes are symbionts on sea anemones (Mariscal, 1970a; 1970b; 1972; Allen, 1972; Stevenson, 1963) and more than one species of anemonefish has been reported to inhabit the same host anemone in nature (Hattori, 1995).

2.2.1. Habitat selection

The anemone fishes establish territories around the host sea anemones and lay eggs in the vicinity of the anemones (Allen, 1972 ; Moyer and Bell, 1976 ; Ross, 1978 ; Richardson *et al.*, 1997). Territorial nature of many other species of damselfishes has also been documented (Myrberg *et al.*, 1967 ; Swerdloff, 1970 ; Clarke, 1971 ; Boer, 1980 ; Ebersole, 1980 ; 1985 ; Khoda, 1981 ; 1984 ; Shpigel, 1982 ; Jones and Norman, 1986 ; Allen, 1991 ; Itzkowitz *et al.*, 1995). Eventhough there are competitions for territories in damselfishes, the survival of juveniles is not affected by density (Doherty, 1982). Also it is reported that the recruitment of juveniles is more in coral heads with predominant conspecific population (Sweatman, 1983). They locate and choose preferred sites with conspecifics using dissolved chemical cues (Sweatman, 1988). The active selection of settlement sites by the larvae may be more important to the distribution of recruits than adult aggression (Sweatman and St. John, 1990). Booth (1992) suggested that visual cues also supplement chemical cues in site choice and the habitat preferences of the larvae influence the settlement pattern in a small scale, while settlement in a large scale may be synchronised as a result of oceanographic factors and spawning patterns. Harrington, (1993; 1995) attributed adult aggression also as a factor determining the distribution and the extend of aggression by resident adults on intruding juveniles depends on size, colour pattern and species identity of juvenile recruits. The younger and smaller juveniles select the site and settle in to it more frequently than older and larger ones (Danilowicz, 1997). Settlement preferences influenced the time to reach maturity size and proportion of settlers reaching maturity in one year, compared to random settlement (Booth and Willington, 1998). The complex behaviours exhibited by reef fishes during the transition

period from pelagic to reefal environment determine their distribution pattern (Ohman *et al.*, 1998). The feeding history and condition affected the early survival and patterns of larval arrival at reefs in the damselfish *Stegastes partitus* (Booth and Hixon, 1999). After settlement, the size of territory defended by individual fishes is related to the length of fishes (Letourneur, 2000).

2.2.2. Symbiosis

The association of anemonefishes and sea anemones has been a subject of interest to marine biologists for over a century. This was first observed by Collingwood in 1868 who identified some 'connexion' between the fish and the sea anemone (Fautin, 1991). Since then various aspects of this association, such as benefits to host and fish, mechanisms of host location, specificity and biochemical aspects have been the subjects of extensive studies. Apart from the anemonefishes (*Amphiprion* spp.), other pomacentrids such as *Dascyllus* spp. (Stevenson, 1963) and some shrimps such as *Periclimenes* spp. (Guo *et al.*, 1996) also live in association with sea anemones. There are 28 species of anemonefishes and 10 species of symbiotic sea anemones (Allen, 1991). In the absence of sea anemones under captive conditions the fishes establish and defend territories in algal turfs, holes, air bubbles etc., and exhibit similar behaviour as observed in real anemones (Mariscal, 1970b; 1972).

Mariscal (1970b) reported that both the fishes and anemones are benefited by the association and hence it can be termed as mutualism. The benefits accrued by the fishes from the association are protection from predators, tactile stimulation from anemone tentacles, reduced susceptibility to various diseases and chances to feed anemone tissues, waste materials, and sometimes the symbiotic shrimps of anemones (Mariscal, 1970b). The advantages for the host anemones include protection from predators especially

chaetodontids (Mariscal, 1970b; Godwin and Fautin, 1992), removal of necrotic tissue, tactile stimulation, removal of organic and inorganic material from and around the sea anemone, possible removal of anemone parasites and the provision of food by some species of anemonefishes (Mariscal, 1970b). But feeding the anemones by the fish may be a phenomenon seen in aquaria only since the natural diet of anemonefishes is mainly zooplankton and algae which they never take to the territories (Allen, 1972). Also the fish lay eggs on to submerged objects close to their host anemones (Allen, 1972; Moyer and Bell, 1976; Ross, 1978).

The mechanism of protection from sea anemones had been subjected of intensive studies and various theories were suggested. Schlichter (1972) reported the presence of protective substances secreted by the sea anemones, a coating of which will be formed on objects which are in constant touch with them. Acclimated anemonefishes thus escape the sensory mechanism of the anemones and do not induce nematocyst discharge. But later it was found that protection ability of anemonefish mucus is not derived from the anemone mucus as inhibitory factors, instead the mucus of anemonefishes having non-stimulatory properties for nematocyst discharge (Lubbock, 1980). Elliott *et al.* (1994) suggested that anemonefishes that are innately protected from the natural host sea anemones do not produce a mucus coating that is biochemically similar to the anemone mucous in naïve condition but they acquire anemone substances in their mucus coat after their association with the host. Resistance to toxins isolated from different species of anemones varied among different species of anemonefishes and is not an essential factor in anemonefish symbiosis (Mebs, 1994). Hatchery reared anemonefishes were not stung by initial contact with their natural host anemones in field experiments, and thus they are protected without acclimation process (Elliott *et al.*, 1995). Eventhough the eggs of anemonefishes are protected from most sea anemones, both natural hosts and others, the larvae are vulnerable to the nematocysts. Protection is developed only at the time of metamorphosis (Elliott and Mariscal, 1996). The anemonefishes are innately protected from their natural host

anemones and can achieve protection from other anemones after acclimation (Elliott and Mariscal, 1997). Juvenile anemonefishes detect their host sea anemones by olfactory cues and this ability is achieved as a result of olfactory imprinting to the host anemone by the fish at embryonic stage (Arvedlund and Neilsen, 1996; Arvedlund *et al.*, 1999). But *Amphiprion melanopus* did not imprint to unnatural host anemone and it was suggested that the innate preference to the natural host was enhanced by imprinting (Arvedlund *et al.*, 1999). The mechanism of protection differs among species of anemonefishes, and in different combinations of anemonefishes and anemones. The more generalist fishes that associate with more number of anemones (eg. *A. clarkii*) find and adapt to host by virtue of behaviour and host specialists (eg. *Premnas biaculeatus*) locate host by chemical cues (Fautin, 1991).

2.2.3. Feeding

Pomacentrids are generally omnivorous feeding on algae, zooplankters and a wide variety of other invertebrates (Allen, 1991). The gut analysis of anemonefish was done by Allen (1972) who reported the occurrence of algae and zooplankters. The diet of four species of *Chromis* comprised maily of copepods and larvaceans (Tribble and Nishikawa, 1982) and that of *Chromis notata* pelagic tunicates and copepods (Ochi, 1985c). Coughlin (1990) reported that the use of specialized feeding behaviour and the ability to vary feeding behaviour are adaptations for feeding evasive prey such as copepods. Pillai and Madanmohan (1990) reported that the damselfish *Abudefduf glaucus* is a herbivore. Certain species such as *Parma victoriae* (Jones and Norman, 1986), *Stegastes nigricans* (Galetto and Bellwood, 1994) and *Pomacentrus wardi* (Sale, 1976) are also herbivores. Letourneur *et al.*, (1997) stated that adult *Stegastes nigricans* is mainly an algal feeder, whereas zooplankters were seen in the gut of smaller fishes.

2.3. Social organization

Damselfishes exhibit distinct social behaviour. Reports on the social organization for various species have been given in earlier accounts (Swerdloff, 1970 ; Myrberg *et al.*, 1967 ; Clarke, 1971 ; Ochi, 1986a ; Ochi and Yanagisawa, 1987, Ochi, 1989b ; Hirose, 1995). Clarke (1971) studied the social behaviour of the Garibaldi, *Hypsypops rubicundus* and stated that the adults were territorial throughout the year and males were more strict in territoriality. Moyer and Bell (1976) and Ochi (1989a) reported that in the Japanese populations of the anemone fish *Amphiprion clarkii*, there was usually a single dominant pair and a varying number of subdominant non breeders in a colony. Only the dominant fishes were sexually active. The social hierarchy in anemone fishes is achieved by aggressive dominance by the larger fishes (Fricke and Fricke, 1977). There is considerable difference in the growth of 0 – year old anemone fishes and this is due to growth inhibition by larger early settlers on late settlers (Ochi, 1986a). The size interval between fishes diminished with increasing number of fishes in the same host ; the lengths of female, male and largest sub adult were more in such cases (Fautin, 1992). In damselfishes, the aggression by resident adults towards settling juveniles reduced after continued habituation to their presence (Harrington, 1995). The settled adult males vacated old territories if newer high quality territories were available and quickly occupied them (Itzkowitz *et al.*, 1995). The colonies of *Dascyllus reticulatus* usually comprised of 3 to 7 individuals and each group contained 1 to 2 males and several females all of which were smaller than the smallest resident male (Schwarz, 1995). The structural complexities of the habitat and the behavioural differences in habitat use among species is an important factor for the reef fish biodiversity (Ormond *et al.*, 1996). The distribution and the size of wild population of damselfishes are controlled by post-settlement processes such as substrate type, competition for space and availability of food (Nemeth, 1997).

2.4. Reproduction

The reproductive patterns and behaviour of various species of pomacentrids have been described from wild populations in different parts of the world. Studies have also been made on the sexuality and reproductive tactics such as hermaphroditism in some species.

2.4.1. Social control on maturity

Studies on the social behaviour and reproduction have been done on a number of pomacentrids (Myrberg *et al.*, 1967 ; Swerdloff, 1970 ; Clarke, 1971 ; Ochi, 1986b ; Ochi and Yanagisawa, 1987). Sex reversal, one of the major aspects of reproduction is a result of the social interactions of fishes (Shapiro, 1984). He concluded that there should be a minimum number of fishes of the initial sex to induce a sex change in one of them and this number varied for different species. Female damselfishes use sounds produced by conspecific males to locate males and nesting sites (Myrberg *et al.*, 1986). In anemonefishes, the onset of breeding is not only determined by the age but also by the ranking in the social hierarchy (Ochi, 1986a). The sex change in anemone fishes occurs only as the best of a bad situation, thus a male changes sex only when it does not get a female partner (Ochi and Yanagisawa, 1987). For breeding males which lost mates, there will be a reproductive pause due to the time taken for sex change and for the unmated males the adaptability is lost by transformation to female (Ochi, 1989a). Largest non-breeders refrained from becoming females to keep their gonads ambisexual so that they could replace either of a breeding pair. Breeding spaces are available to non-breeders only after the disappearance of one or both members of the established pairs (Ochi, 1989b). Size composition of the members in a colony and mobility also affect the pattern of pair formation in anemonefishes (Hirose, 1995). Due to the difference in timing among individuals in the development of ovarian tissues of the

hermaphroditic gonads, different life history pathways have been reported in anemone fish *Amphiprion clarkii* (Hattori and Yanagisawa, 1991).

Ross (1990) suggested that the direction of sex change is determined by the mating system and the predicted direction of sex change for polygynous fishes is from female to male. Polygynous mating system with protogynous sex change is observed in *Dascyllus reticulatus* in which larger individuals in a colony are usually males (Schwarz and Smith, 1990; Schwarz, 1995). Godwin (1995) suggested the effect of ecological factors and phylogenic history in the mating system of humbug damselfishes. The females in a group of *D. reticulatus* maintained size based dominance hierarchy among themselves but did not defend individual territories within their residence coral (Schwarz, 1995). The frequency of spawning and territorial defense is more when population density is high in *Chromis dispilus* (Barnett and Pankhurst, 1996) and the frequency of agonistic behaviour will be more in captivity than in natural conditions (Cleveland, 1999).

2.4.2. Sexuality and hermaphroditism

Pomacentridae includes gonochorists, with predetermination of sex, protogynous and protandrous hermaphrodites, and bisexual types in which the gonadal primordium bears both types of gonia (Fishelson, 1998). Protandrous nature of anemonefishes has been reported by Fricke and Fricke (1977), Moyer and Nakazono (1978), Brusle–Sicard and Reinboth (1990), Hattori and Yanagisawa (1991), Brusle–Sicard *et al.* (1994) and Godwin (1995). Godwin and Thomas (1993) studied the changes in steroid profiles during sex reversal in *Amphiprion melanopus*. Gonadal structure and the distribution of steroid producing cells in the gonad of *Amphiprion frenatus* was described by Nakamura *et al.* (I994). Protogyny in *Dascyllus reticulatus* has been reported by Schwarz and Smith (1990) and Schwarz (1995). Tzioumis and Kingsford (1999) reported that the damselfish *Parma microlepis* is gonochoristic. The phenomenon of

reverse sex change has been reported in certain protogynous fishes like pomacanthids, gobiids and epinephelids when their ranking in the hierarchy is lowered (Kuwamura and Nakashima, 1998). Asoh *et al.* (2001) described the non – functional protogynous gonadal development in *Dascyllus albisella* where the transition to maleness was assumed to have occurred after the onset of cortical alveolus stage, but before the final oocyte maturation and spawning as females.

Reproductive biology and population characteristics of some species have been studied from Indian waters also (Madanmohan *et al.*, 1987; Pillai *et al.*, 1987b; Pillai and Madanmohan, 1990; Gopakumar *et al.*, 1991; Vijayanand, 1994; Murty, 2002).

2.4.3. Breeding patterns

Reports on the spawning behaviour and spatial and temporal patterns are available for many species of pomacentrids, such as *Chromis multilineata* (Myrberg *et al.*, 1967), *C. caeruleus* (Swerdloff, 1970), *C. cyanea* (Boer, 1980), *C. notatus* (Ochi, 1986a), *C. dispilus* (Tzioumis and Kingsford, 1995), *Hypsipops rubicundus* (Clarke, 1971 ; Sikkel, 1988 ; 1989 ; 1994a ; 1994b; 1995a ; 1995b ; Knapp *et al.*, 1995), *Amphiprion clarkii* (Moyer and Bell, 1976 ; Ochi, 1985b ; 1989a; 1989b), *A. melanopus* (Ross, 1978), *A. latezonatus* (Richardson *et. al.*, 1997), *A. akindynos* (Richardson *et al.*, 1997), *Acanthochromis polyacanthus* (Thresher, 1983 ; Thresher and Moyer, 1983 ; Kavanagh, 2000), *Abudefduf saxatilis* (Mochek, 1978 ; Prappas *et al.*, 1991 ; Foster, 1987), *A. troscheli* (Foster, 1987), *A. abdominalis* (Tyler and Stanton, 1995), *Stegastes altus* (Khoda, 1988), *S. partitus* (Knapp, 1993 ; Knapp *et al.*, 1995), *S. lucostictus* (Knapp *et al.*, 1995), *Amblyglyphydodon leucogaster* (Goulet, 1994 ; 1995 ; 1997 ; 1998), *Dascyllus albisella* (Danilowicz, 1995a ; 1995b), *D. marginatus* (Fricke, 1980), *Parma microlepis* (Tzioumis and Kingsford, 1995), *Microspathodon chrysurus* (Pressley, 1980), *Plectroglyphydodon johnstonianus* (Mc Donald, 1976). The effect of hormones in the reproductive behaviour and breeding was described by Hobby and

Pankhurst (1997), Pankhurst (1990; 1995), Pankhurst and Barnett (1993), Pankhurst and Carragher (1995) for *Chromis dispilus*, Pankhurst *et al.* (1999; 2000) for *Acanthochromis polyacanthus* and Sikkel (1993) for *Hypsipops rubicundus.*

Many pomacentrid species exhibit lunar periodicity in spawning (Ross, 1978 ; Foster, 1987 ; Richardson *et al.*, 1997). There are also a number of species which do not show any lunar cyclic spawning (Mochek, 1978; Pressley, 1980 ; Ochi, 1986b ; Foster, 1987). Females of many damsel fishes usually selected nests with younger stage eggs for spawning (Sikkel, 1988 ; 1989 ; 1994b ; Knapp *et al.*, 1995 ; Goulet, 1994 ; 1997). Nest quality is also a factor determining the spawning site choice by females (Sikkel, 1995b). Promiscuous mating system was observed for *Amblyglyphydodon leucogaster* females and are capable of laying new batch of eggs every second day (Goulet, 1994 ; 1997). Knapp and Kovach (1991) and Knapp and Warner (1991) reported that courtship rate influenced spawning site choice by female *Stegastes partitus.* Females of many species selected nests of males with higher egg survival (Knapp, 1993; Peterson, 1995).

Most of the accounts on reproductive behaviour of pomacentrids show that the parental care is exclusively done by the males till the hatching of the eggs. Biparantal care is reported from *Acanthochromis polyacanthus* (Kavanagh, 2000). Filial cannibalism by custodial males is reported from certain species (Hoelzer, 1988 ; 1995 ; Sikkel, 1994a). Eggs of all pomacentrids hatch in the night, mostly a few hours after sunset except in *Acanthochromis polyacanthus* where mid day hatching is reported (Kavanagh, 2000). McAlary and McFarland (1993) reported hatching failure to the eggs of *Abudefduf saxatilis* when they were exposed to continuous light during their twilight hatching period whereas enhanced hatching occurred when they were kept in dark. Diel periodicity of hatching of eggs of *Hypsypops rubicundus* was descried by Alcalay and Sikkel (1994).

2.5. Larval development and rearing

Most pomacentrids have pelagic larval life prior to their settlement. Thresher *et al* (1989) estimated the duration of larval stage in Pacific damselfishes and reported that the mean planktonic duration varied between 0 and 37.4 days. Wellington and Victor (1989) estimated the larval duration in 100 species of Pacific and Atlantic damselfishes from daily growth increments on otoliths of juveniles. The growth after settlement was faster than the pre settlement growth in two species of pomacentrids, *Pomacentrus coelestis* and *Chromis atripectoralis* (Thorrold and Milicich, 1990). The osteological development of larvae and juveniles of the yellow tail damselfish *Microspathodon chrysurus* was described by Pothoff *et al.* (1987). Alshuth *et al.* (1998) described the larval development of laboratory reared sergeant major *Abudefduf saxatilis*. The eggs and larvae of 52 species of pomacentrids under tank breeding conditions were reported by Tanaka (1998).

Different species of anemonefishes have been reared in different parts of the world (Ballard, 1976; Alava and Gomes, 1989; Allen, 1991; Moe, 1992; Hoff, 1996; Maroz and Fishelson, 1997; Wilkerson, 1998; Johnson *et al.*, 1998) and in India by Gopakumar *et al.* (1999) and Ignatius *et al.* (2001). Alayse (1984) reported increased survival rates when larvae of *A. ocellaris* were fed with enriched feed. Coughlin *et al.* (1992) and Coughlin (1993) studied the prey location and searching behaviour of *A. perideraion* larvae. The effect of dry feed and age at weaning of larvae and juveniles of *A. percula* was studied by Gordon *et al.* (1998). Arvedlund *et al.* (2000) recommended a 16 hour light and 8 hour dark regime as ideal for the rearing of *A. melanopus* larvae. Green and McCormick (2001) studied the ontogeny of digestive system of *A. melanopus* and found out that the alimentary tract changes rapidly throughout the larval period. There are only fewer reports on the rearing of other damselfishes (Danilowicz and Brown, 1992; Moe, 1992; Job *et al.*, 1997; Alshuth *et al.*, 1998).

3. A SYSTEMATIC ACCOUNT OF COMMON POMACENTRIDS

3.1. Introduction

Pomacentrids constitute one of the largest groups of reef fishes which include more than 300 species. They are generally small in size, rarely exceeding a length of 10 to 15 cm. There is a wide range of colouration from black, brown and grey to bright shades of orange, yellow, red and blue. They are characterised by ovate to elongate and laterally compressed body shape, a single dorsal fin composed of 8 to 15 spines and a number of flexible , segmented rays and an anal fin containing two spines and a variable number of segmented rays. The head, body and fin bases are covered with medium sized scales that usually have microscopic serrations along the exposed margins. The jaw teeth are arranged in one or two rows and range from a sturdy conical shape to a flattened columnar or spatulate form. Recently, there have been a number of books and technical papers that deal with the pomacentrid fauna of specific regions. The major works include those of the Japanese Archipelago by Masuda *et al* (1984), the Central and Western Pacific Ocean by Allen (1975), the Red Sea by Allen and Randall (1980), the Western Indian Ocean by Smith (1960) and Southern Africa by Allen (*In* Smith and Heemstra, 1986). The classification of anemonefishes by Allen (1972) was the first attempt exclusively on this group. The most recent and accepted classification of the family Pomacentridae is by Allen (1991). Reports on the colour polymorphism in various species of anemonefishes were published by Bell *et al.* (1982), Marliave (1985) and Richardson (1998). Eventhough pomacentrids are abundant in Indian waters, much attention has not been paid to study their taxonomy, distribution and abundance. Jones and Kumaran (1980) described the pomacentrids from Laccadive Archipelago in their book '*Fishes of Laccadive Archipelago*' which still remains to be the authentic account of pomacentrid taxonomy from Indian waters.

3. 2. Materials and methods

The fishes for the study were collected from Vizhinjam, Minicoy Island (Lakshadweep) and Rameswaram. The specimens were collected in live condition to study their natural colour patterns. After noting the colour patterns, they were sacrificed and preserved for morphometric and meristic analysis. All the measurements were taken to the nearest millimeter. The observations were compared with the earlier descriptions on the group. Identification of species was made based on Allen (1991). SL indicates standard length and HL indicates head length.

3. 3. Results

Twenty four species belonging to nine genera were identified in the present study. Detailed meristic counts are presented in Table 1 (Page 48).

1. Genus *Abudefduf* Forskal, 1775

They are deep bodied fishes with distinctly forked caudal fins. Colour pattern is characterized by alternate vertical light and dark bands, the number, width and position of bands vary with species. Often grows to larger sizes of more than 100 mm (SL). Margin of sub orbital and pre opercle smooth. Generally non - aggressive in habit and thus many species are suitable for marine community aquaria.

1. *Abudefduf bengalensis* (Bloch) 1787 - Bengal sergeant

Plate : 1a

Chaetodon bengalensis Bloch 1787
Labrus macrogaster Lacepede 1802
Glyphidodon affinis Günther 1852
Glyphisodon palmery Ogilby 1913

Characterized by seven black transverse bands. Width of dark bands lesser than the white bands. The first band along the nape, second from the origin of dorsal fin, third from fifth or sixth dorsal ray, fourth from ninth dorsal ray, fifth from beginning of soft dorsal, sixth from the end of dorsal fin and the seventh across the caudal peduncle. Scales sometimes yellowish with bluish edges. Caudal fin and soft portion of dorsal and anal fins usually dark. Depth 1.7 in SL. Head length 3.3 - 3.3 (SL), base of dorsal fin 1.6 (SL). Eye diameter 2.9 – 3.4 (HL), inter orbital width 2.3 – 2.6 (HL), snout length 3.9 – 4.6(HL), anal fin base 1.1 - 1.2(HL), depth of caudal peduncle 1.6 – 1.7 (HL).

Number of specimen examined : 6
Size range of specimens : 71 – 90 mm SL.
Collection site : Vizhinjam.

2. *Abudefduf notatus* (Day) 1869 - Yellow tail sergeant

Plate : 1b

Glyphidodon notatus Day 1869
Abudefduf clarkii Snyder 1911
Indoglyphidodon abboti Fowler 1944
Chrysiptera paucifasciata Fowler 1946

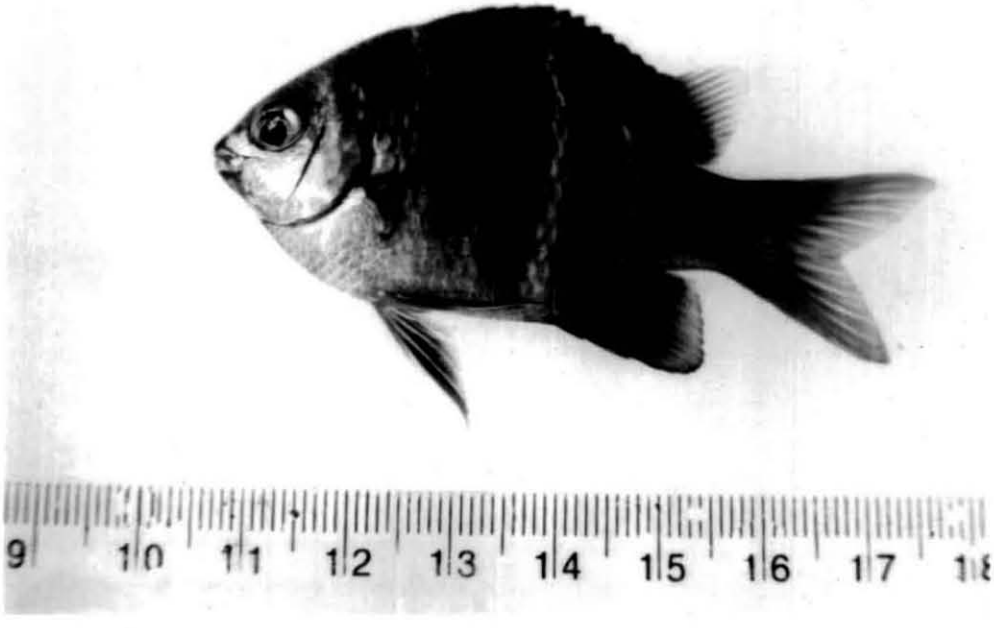
Body dark grey in colour with five thin white transverse bands. The middle band is more prominent and is 2 – 3 scales in width. A dark spot at the origin of lateral line and another dark blue spot at the upper part of pectoral fin base. Depth 1.8 in SL. Head length 3.3 (HL) and base of dorsal fin 1.6 (SL). Inter orbital width 2.6 (HL), snout length 4.2 (HL), anal fin base 1.2 (HL), and caudal peduncle depth 1.8 (HL).

Number of specimen examined : 1
Size of specimen : 51 mm (SL)
Collection site - Vizhinjam.

Plate 1

a. *Abudefduf bengalensis*

b. *Abudefduf notatus*



3. *Abudefduf septemfasciatus* (Cuvier) 1830 - Banded sergeant

Plate : 2a

Glyphisodon septemfasciatus Cuvier 1830
Abudefduf multifasciatus Seale 1906

Body with seven vertical black bands, first one originating just behind the eye, second behind the opercle, third between third and fifth dorsal spines, fourth between seventh and ninth dorsal spines, fifth between tenth and twelfth dorsal spines, sixth at the origin of soft dorsal and the seventh at the origin of caudal peduncle. Dark blotch present at the upper part of pectoral fin base. Body depth 1.8 (SL), head length 3.18 (SL) and dorsal fin base (1.8). Eye diameter 4.0 (HL), inter orbital width 2.0 (HL), snout length 4.0 (HL), anal fin base 1.3 (HL) and caudal peduncle depth 1.9 (HL).

Number of specimen examined : 1
Size of specimen : 120 mm (SL)
Collection site : Vizhinjam.

4. *Abudefduf sordidus* Forskal 1775 - Black spot sergeant

Plate : 2b

Chaetodon sordidus Forskal 1775
Glyphidodon leucopleura Day 1877
Abudefduf tridentatus Clark 1938

Pale grey in colour with six narrow transverse white bands. The white bands were not distinct in some specimens . A prominent dark blotch on the upper part of caudal peduncle extending to the end of soft dorsal base. Depth 1.6 – 1.7 (SL), head length 3 – 3.1 (SL) and dorsal fin base 1.6 (SL). Eye diameter 3.8 – 4.4 (HL), inter orbital width 2.4 – 2.7 (HL), snout length 4.1 – 5.4 (HL), anal fin base 1.5 – 1.6 (HL) and caudal peduncle depth 1.9 (HL).

Number of specimens examined : 5
Size range of specimens : 103 – 148.mm (SL)

Collection site : Vizhinjam.

5. *Abudefduf vaigiensis* (Quoy and Gaimard) 1825 - Indo Pacific sergeant

Plate : 2c

Glyphisodon vaigiensis Quoy and Gaimard 1825
Chaetodon trywhitti Bennet 1834
Glyphisodon rahti Cuvier 1830
Glyphysodon quadrifasciatus Bleeker 1847
Glyphisodon binair Montrouzier 1857
Abudefduf quinquelineatus Von Bonde 1934
Abudefduf caudobimaculatus Okada and Ikeda 1939

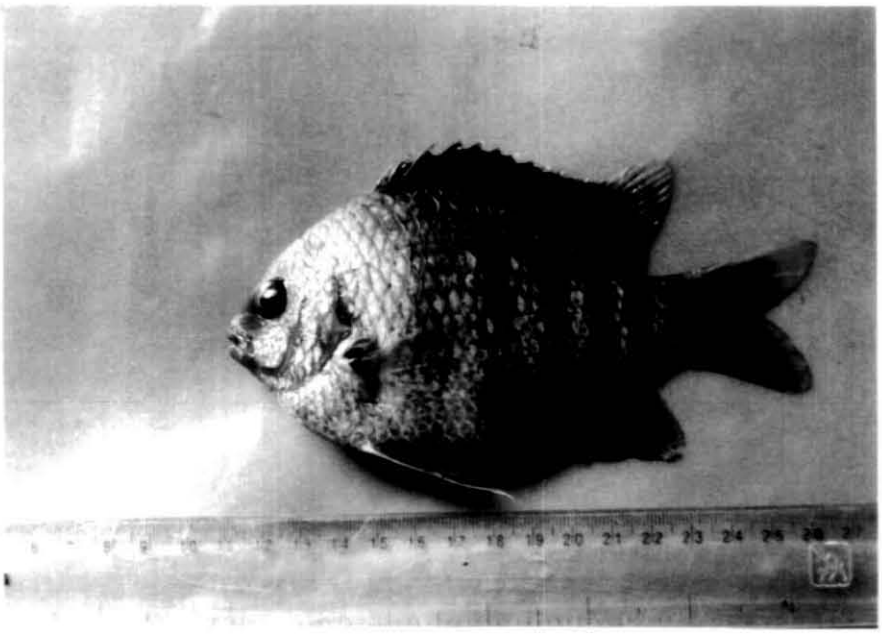
Five dark bands across the body. Dorsal part of the body above lateral line from forehead to origin of soft dorsal golden yellow in colour. The black bars are slightly narrower than the inter spaces. A dark blue spot at the upper part of the pectoral fin base. Caudal fin and edges of soft dorsal and anal dark. Body depth 1.8 – 1.0 (SL), Head length 3.2 – 3.8 (SL), dorsal fin base 1.7 – 1.8 (SL). Eye diameter 2.8 – 3.5 (HL), inter orbital width 2.2 – 2.9 (HL), snout length 3.1 – 3.8 (HL), anal fin base 1.3 – 1.7 (HL) and caudal peduncle depth 1.4 – 2.1 (HL).

Number of specimen examined : 18
Size range of specimens : 58 – 115 mm (SL)
Collection site : Vizhinjam.

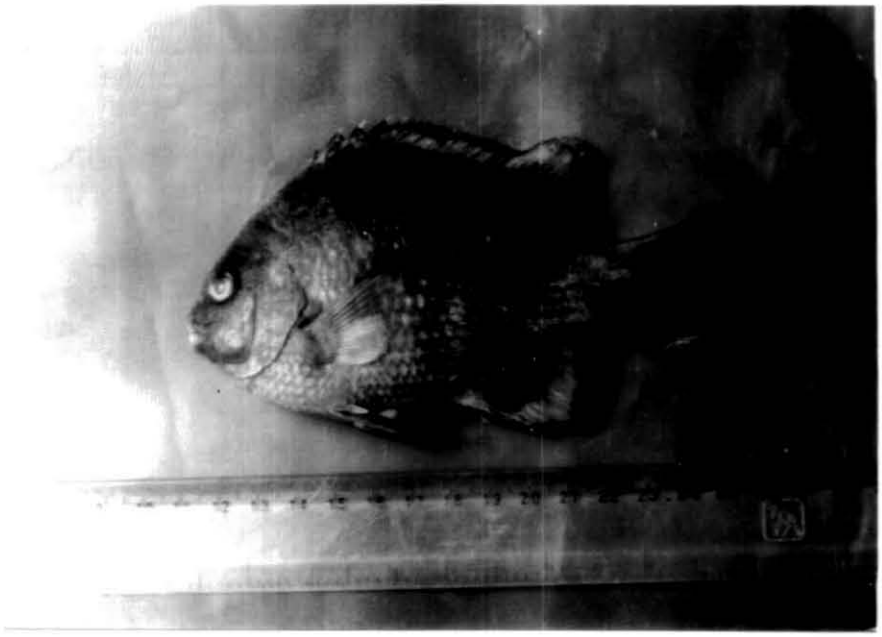
Earlier this species was described as *Abudefduf saxatilis*. But the difference in the position of the fourth black bar between Atlantic and Indo Pacific specimens is the distinguishing character between these two species. The Atlantic specimen in which the fourth bar originates directly below the last dorsal spine is regarded as *A. saxatilis* and the Indo Pacific specimen in which the fourth bar originates from the middle of soft dorsal fin is *A . vaigiensis*. Both are sometimes considered as subspecies (Allen, 1991). The fourth bar of all the specimens examined in the present study originated from the base of fourth to fifth dorsal ray.

Plate 2

a. *Abudefduf septemfasciatus*



b. *Abudefduf sordidus*



c. *Abudefduf vaigiensis*

2. Genus *Amphiprion* Bloch and Schneider, 1801

Commonly known as the clownfishes or anemonefishes due to their association with sea anemones. Opercle, sub opercle, and inter opercle with strong serrations. Scales small and more than 40 longitudinal rows from opercle to caudal fin base.

1. *Amphiprion sebae* Bleeker 1853 - Sebae anemonefish.

Plate : 3

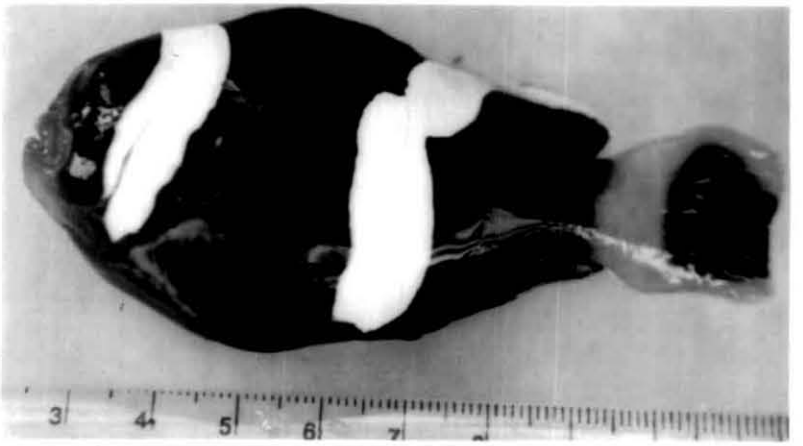
Dark brown body with two white bars, the first bar between eye and operculum and the second bar originates from ninth or tenth dorsal spine. Width of second bar 14 – 17 scales at lateral line and it may continue posteriorly along distal edge of soft dorsal. Snout, ventral portion upto pelvic fin, distal edges of soft dorsal, anal and usually pectoral fins pale. Caudal peduncle and caudal fin pale entirely or with a dark spot on caudal fin or sometimes dark with light margins. Body depth 2.1 – 2.3 (SL), head length 3.0 – 3.5 (SL) and dorsal fin base 1.7 – 1.9 (SL). Eye diameter 3.0 – 3.4 (HL), inter orbital width 2.7 – 3.2 (HL), snout length 3.3 – 3.8 (HL), anal fin base 1.2 – 1.3 (HL), and caudal peduncle depth 1.8 – 1.9 (HL).

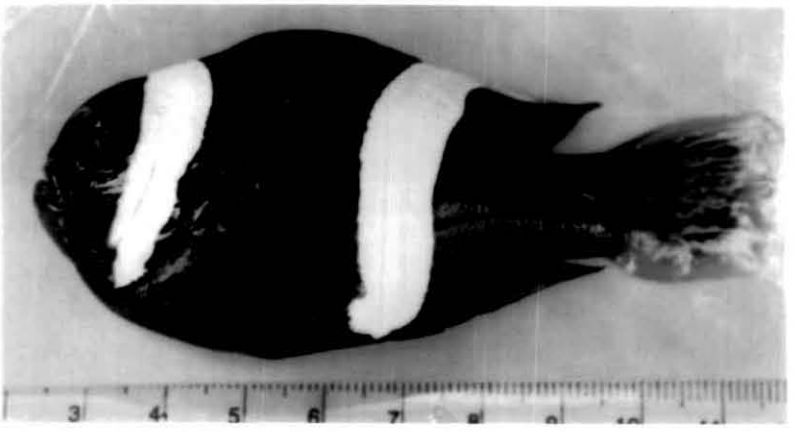
Number of specimens examined : 31
Size range of specimen : 33 - 94 mm (SL)
Collection site : Rameswaram.

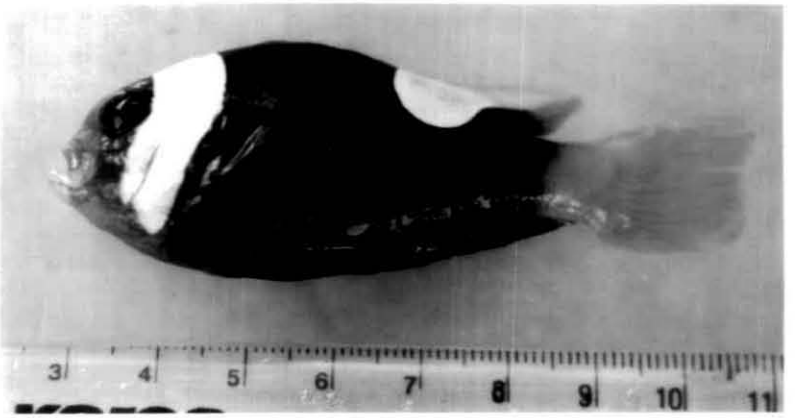
Many of these characters closely match to other species of anemonefishes such as *A. chrysogaster, A. clarkii* and *A. polymnus.* For *A. clarkii* and *A. chrysogaster* the third white bar is present on the caudal peduncle. But for *A. clarkii* the dark caudal fin or dark blotch on caudal fin are unusual. Although *A. polymnus* possesses a prominent triangular dark blotch on the caudal fin, the second bar will be usually incomplete in this species. But all the specimens in the present study possessed two white bars which are complete in most cases. Also the black colouration in the caudal fin was noted in many of the specimens in the present study with varying intensity. Allen (Personal

Plate 3. *Amphiprion sebae*

Different colour variants of the species







communication) opined that such colour variations are usual for *A. sebae* and hence they are regarded to be the same species. In experimental breeding tanks, individual fishes with the above varying characters became pairs and bred. The offsprings from the same parents also were distinctly variable in colour pattern even after attaining maturity.

3. Genus *Chromis* Cuvier, 1814

Smaller fishes mainly inhabiting coral seas. Preopercle and opercle smooth, sub orbital hidden by scales. A pair of procurrent spines on the upper and lower edge of caudal fin base. Teeth biserial. Many members under this genus are brightly coloured and are important as ornamental fishes.

1. *Chromis viridis* (Cuvier) 1830 - Blue green chromis

Plate : 4a

Pomacentrus viridis Cuvier 1830
Heliastes lepisurus Cuvier 1830
Heliastes frenatus Cuvier 1830
Dascyllus cyanurus Rüppel 1835
Glylphisodon bandanensis Bleeker 1851

Previously referred to as *Chromis caeruleus*. Greenish dorsally and silvery colour in the lower half. Scales usually have a blue spot. Body depth 2.3 - 2.4 (SL), head length 3.1 – 3.4 (SL) and dorsal fin base 2.0 – 2.4 (SL). Eye diameter 3.3 – 3.4 (HL), inter orbital width 2.6 – 2.8 (HL), snout length 4.0 – 4.3 (HL), anal fin base 1.5 – 1.6 (HL) and caudal peduncle depth 2.0 – 2.2 (HL).

Number of specimens examined : 4
Size range of specimens : 43 – 55 mm (SL)
Collection site : Minicoy.

4. Genus *Chrysiptera* Swainson, 1839

Smaller bodied fishes inhabiting rocky areas and coral seas. Pre opercle and sub orbital smooth. Notch between pre orbital and sub orbital absent. Teeth biserial. Caudal fin mostly forked. Moderately aggressive especially during spawning and brooding, otherwise peaceful and are suitable for community aquaria.

1. Chrysiptera biocellata (Quoy and Gaimard) 1824 - Two spot demoiselle

Plate : 4b

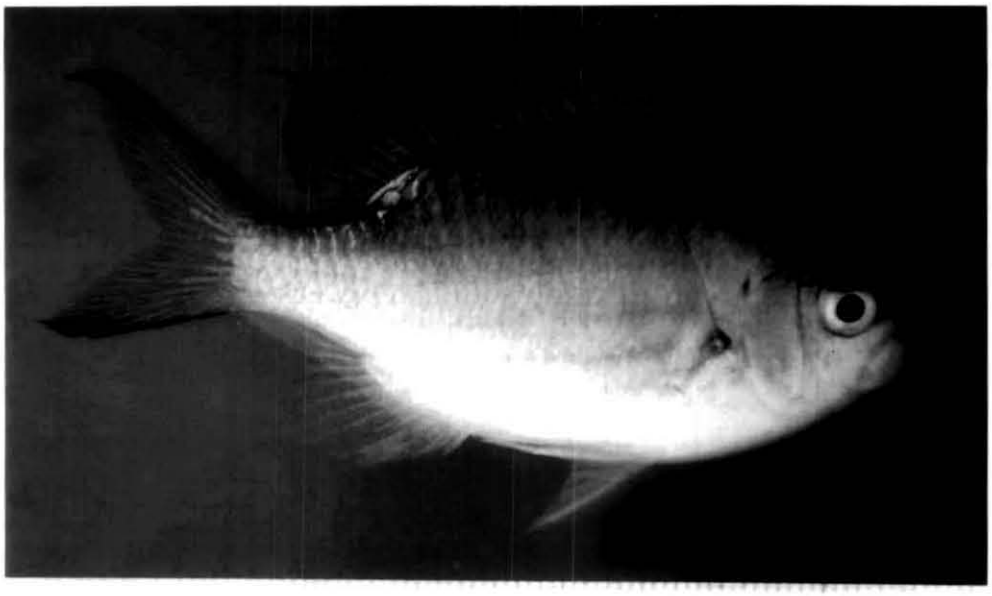
Glyphisodon biocellatus Quoy and Gaimard 1824
Chaetodon brownriggii Bennett 1828
Glyphisodon antjerius Cuvier 1830
Glyphisodon punctulatus Cuvier 1830
Glyphisodon zonatus Cuvier 1830
Glyphisodon baliensis Bleeker 1849
Glyphisodon rossi Bleeker 1854
Glyphidodon antjerius var. *fasciastus* Günther 1862
Glyphidodon cingulatus Kner 1867
Glyphidodon albocinctus Kner 1867
Heliastes cinctus Playfair 1867

Body light brown in colour with one transverse band originating from the base of fourth dorsal spine. Two black spots at dorsal fin base first at the origin and the second at the end of the soft dorsal. Caudal fin pale. Body depth 2.2 (SL), head length 3.3 (SL) and dorsal fin base 1.6 (SL). Eye diameter 3.0 (HL), inter orbital width 3.0 (HL), snout length 4.5 (HL), anal fin base 1.2 (HL) and caudal peduncle depth 1.8 (HL).

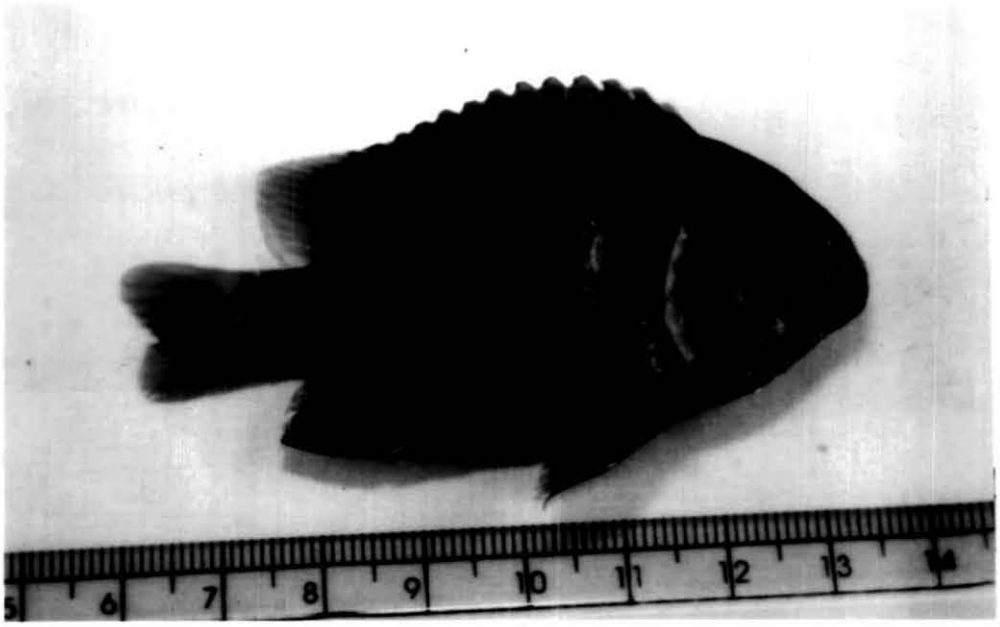
Number of specimen examined : 1
Size of the specimen : 59 mm (SL)
Collection site : Vizhinjam.

Plate 4

a. *Chromis viridis*



b. *Chrysiptera biocellata*



2. *Chrysiptera rollandi* (Whitley) 1961 - Rolland's demoiselle

Plate : 5a

Chromis rollandi Whitley 1961

Anterior part of body brownish and creamy white posteriorly. The dark colour begins at the level of ninth dorsal spine and extends to the level of opercle ventrally. Body depth 2.1 (SL), dorsal fin base 1.6 (SL) and head length 2.9 (SL). Eye diameter 2.7 (HL), inter orbital width 2.7 (HL), snout length 8 (HL), anal fin base 1.6 (HL) and caudal peduncle depth 2 (HL).

Number of specimen examined - 1
Size of specimen - 23 mm (SL)
Collection site - Minicoy.

3. *Chrysiptera unimaculata* (Cuvier) 1830 - One spot demoiselle

Plate : 5b, 5c

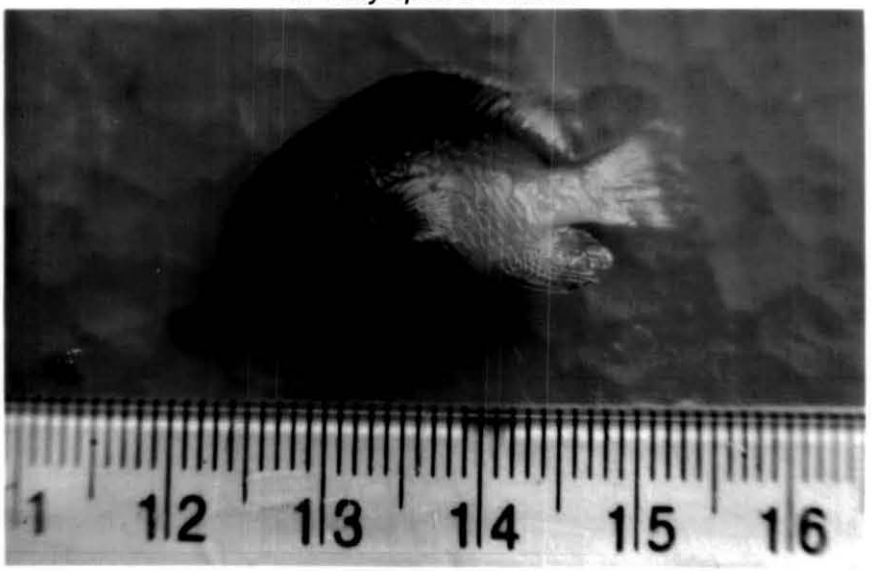
Glyphisodon unimaculatus Cuvier 1830
Glyphidodon dispar Günther 1862
Glyphidodon hemimelas Kner 1868
Glyphisodon filholi Sauvage 1878
Glyphisodon hermani Steindachner 1887

Body light brown with darker anterodorsal region. The demarcation between the anterior darker and posterior lighter regions vary widely among specimens. A dark spot present at the end of soft dorsal base. Pectoral fin usually yellowish. Depth 2.0 – 2.1. (SL), head length 3.4 – 3.7 (SL), dorsal fin base 1.6 – 1.7 (SL). Eye diameter 3.2 – 3.8 (HL), inter orbital width 2.0 – 2.3 (HL), snout length 2.1 – 2.7 (HL), anal fin base 0.9 – 1.1 (HL) and caudal peduncle depth 1.8 – 2.0 (HL).

Number of specimen examined : 10

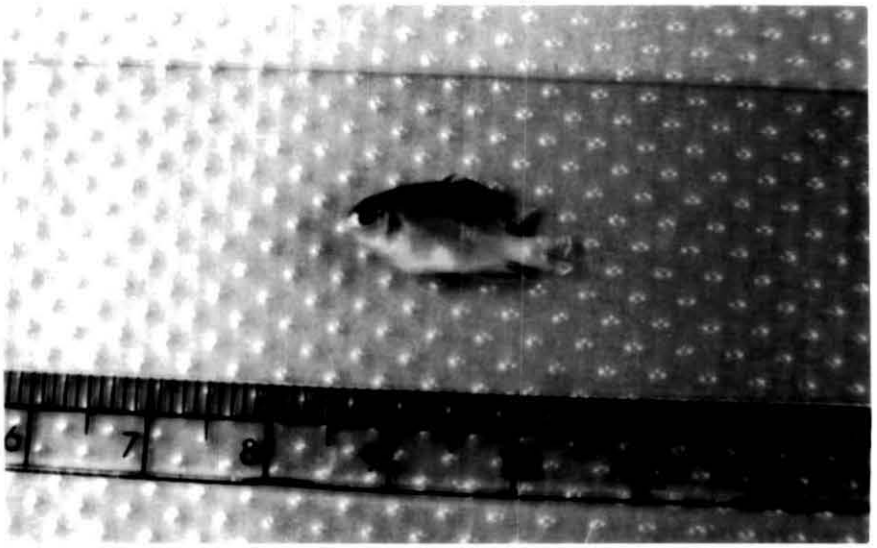
Plate 5

a. *Chrysiptera rollandi*



b. *Chrysiptera unimaculata*

c. *C. unimaculata* juvenile



Size range of specimens : 52 – 65 mm(SL)

Collection site : Vizhinjam.

The colour of juveniles of this species vary significantly from that of adults. The juveniles have two bright blue lines from snout running just above the eye to the posterior part of dorsal fin base. Two black spots with bright blue margins are present at the dorsal fin base, first towards the end of spiny dorsal and the second at the end of soft dorsal. However, the blue lines and the first ocellus disappear as they grow and a single spot without bluish border at the end of dorsal fin base persists in adult.

5. Genus *Dascyllus*

Body deeper and orbicular in shape. Sub opercle and lower edges of sub orbital finely serrate. Two procurrent spines present at the upper and lower edges of caudal fin base. Scales present beyond nostril and chin. Members of this genus are generally aggressive in aquaria.

1. *Dascyllus aruanus* (Linnaeus) 1758 - Humbug dascyllus

Plate : 6a

Chaetodon aruanus Linnaeus 1758
Chaetodon arcuanus Gmelin 1789
Pomacentrus emamo Lesson 1830
Tetradrachmum arcuatum Cantor 1850
Dascyllus blochii Castelnau 1875
Pomacentrus trifasciatus De Vis 1884
Pomacentrus devisi Jordan and Seale 1905
Abudefduf caroli Curtiss 1938

Body white with three black transverse bars. Caudal fin white. Body depth 1.6 – 1.8 (SL), head length 3.3 – 3.4 (SL) and dorsal fin base 1.7 – 1.8 (SL). Eye diameter 2.4 – 2.6 (HL), inter orbital width 2.3 – 2.4 (HL), anal fin base 1.1 - 1.3 (HL), snout length 6.0 – 6.5 (HL) and caudal peduncle depth 1.5 – 2.0 (HL).

Number of specimen examined : 11

Size range of specimens : 33 – 56 mm (SL)
Collection site : Minicoy.

2. *Dascyllus carneus* Fischer 1885 - Indian dascyllus

Plate : 6b

Dascyllus nigripinnis Regan 1908

Body pale to dark grey with a mid dorsal area on both sides reaching slightly below the lateral line. Caudal fin and pectoral fin white. Dorsal, anal and pelvic fins dark. A dark brown line present from the origin of dorsal to the pectoral fin base. Body depth 1.6 – 1.7 (SL), head length 3.3 – 3.5 (SL) dorsal fin base 1.5 (SL). Eye diameter 2.5 – 2.7 (HL), inter orbital width 2.0 – 2.6 (HL), anal fin base 1.1 – 1.2 (HL) and caudal peduncle depth 1.6 – 1.9 (HL).

Number of specimen examined - 5
Size range of specimens - 33 – 56 mm (SL).
Collection site - Minicoy.

This species closely resembles the reticulated damselfish *D. reticulatus* and sometimes referred to by the same name. But the populations of the Indian Ocean from East Africa to Andaman Sea is *D. carneus*, and the Western Pacific form distributed upto Eastern Indian Ocean is *D. reticulatus* (Godwin, 1995). *D. reticulatus* is different in having a dark caudal fin peduncle and caudal fin. Also, *D. carneus* possesses scattered bluish violet spots on the forehead and ventral region (Randall and Allen, 1977).

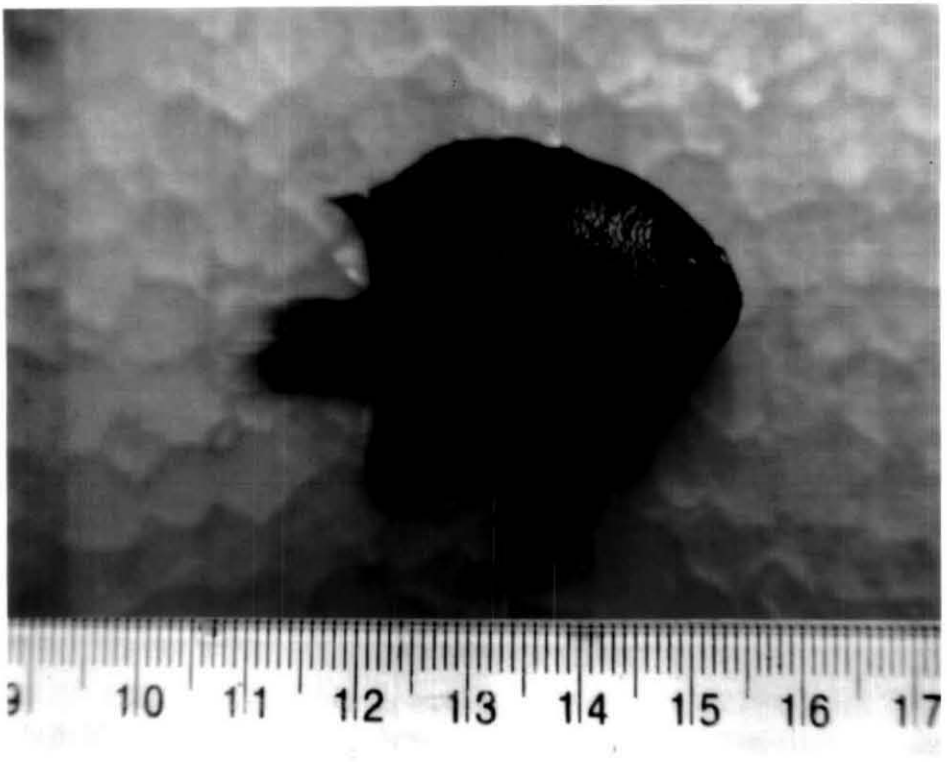
3. *Dascyllus trimaculatus* (Rüppel) 1828 - Three spot dascyllus

Plate : 7a

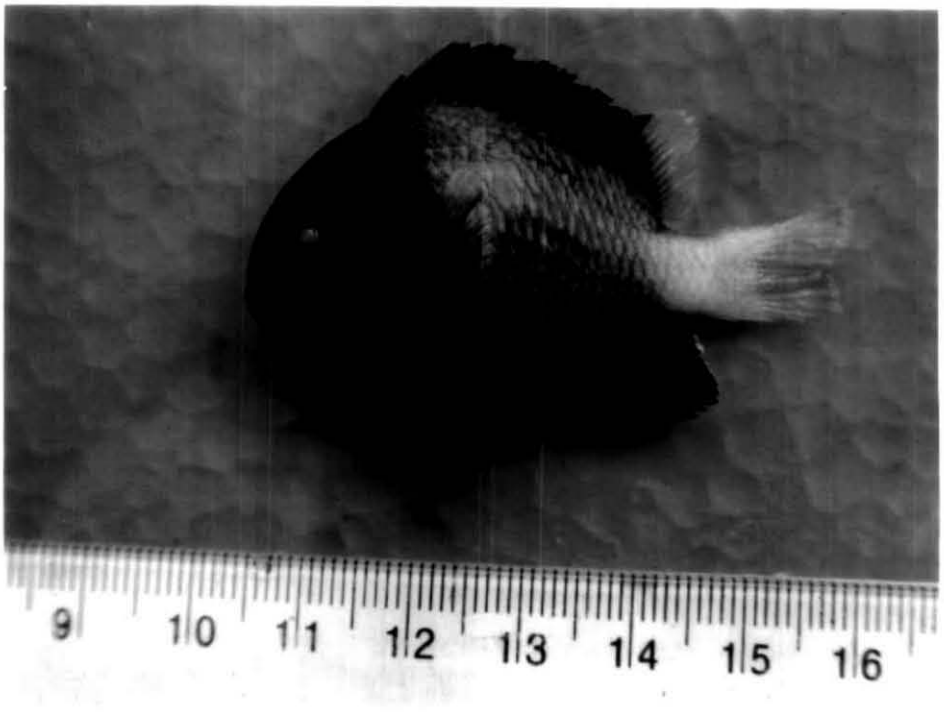
Pomacentrus trimaculatus Rüppel 1828
Pomacentrus nuchalis Bennet 1828
Dascyllus unicolor Bennet 1831
Dascyllus niger Bleeker 1847
Sparus nigicans Gray 1854
Dascyllus axillaris Smith 1936

Plate 6

a. *Dascyllus aruanus*



b. *Dascyllus carneus*



Body dark brown with three white spots, one on forehead and one each mid dorsally on both sides of dorsal fin base. Body depth 1.5 – 1.6 (SL), head length 3.0 – 3.1 (SL) and dorsal fin base 1.6 (SL). Eye diameter 2.6 – 3.4 (HL), inter orbital width 2.2 – 2.8 (HL), anal fin base 1.3 (HL) and caudal peduncle depth 1.9 – 2.1 (HL).

Number of specimen examined : 4
Size range of specimens : 40 – 52 mm (SL)
Collection site : Minicoy

6. Genus *Neoglyphydodon* Allen, 1991

Members of this genus were rare in collections. Resembles to the genus *Chrysiptera* but differs in having a distinctly deeper body. Pre opercle and sub orbital scaled, scales present upto the level of nostrils. Notch between pre orbital and sub orbital absent. Generally less aggressive in aquaria.

1. *Neoglyphydodon bonang* (Bleeker) 1853 - Ocellated damsel

Plate : 7b

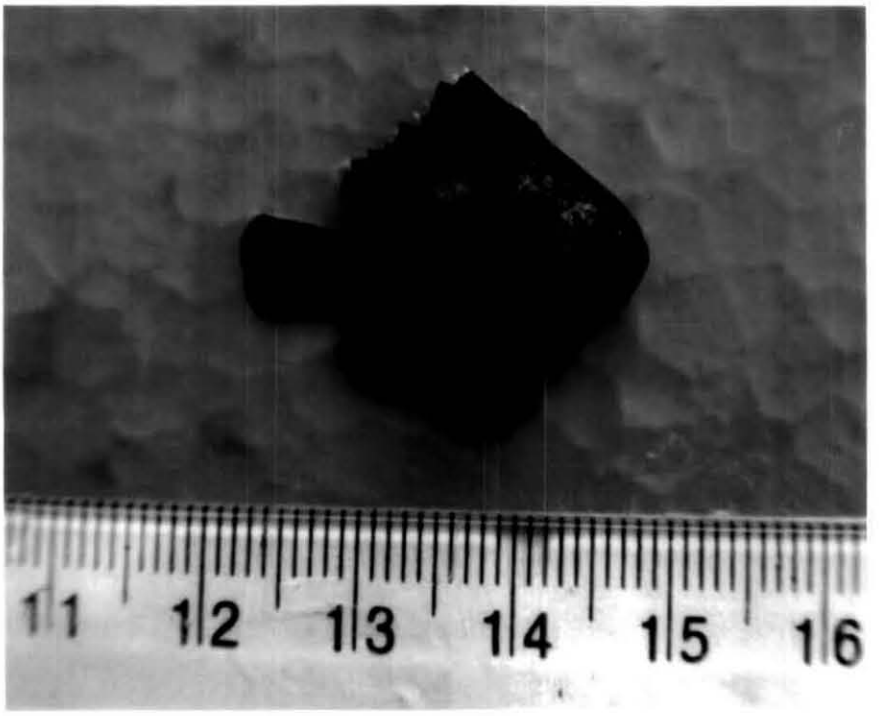
Glyphisodon bonang Bleeker 1853

Body uniform brown in colour. Two black ocelli with blue borders at dorsal fin base, one below tenth and eleventh dorsal spines and the other at the end of soft dorsal extending to caudal peduncle. Body depth 1.8 (SL), head length 3.3 - 3.4 (SL) and dorsal fin base 1.5 (SL). Eye diameter 3.5 – 3.7 (HL), inter orbital width 2.5 - 2.7 (HL), snout length 3.5 – 3.7 (HL), anal fin base 1.0 – 1.1 (HL) and caudal peduncle depth 1.7 – 1.9 (HL).

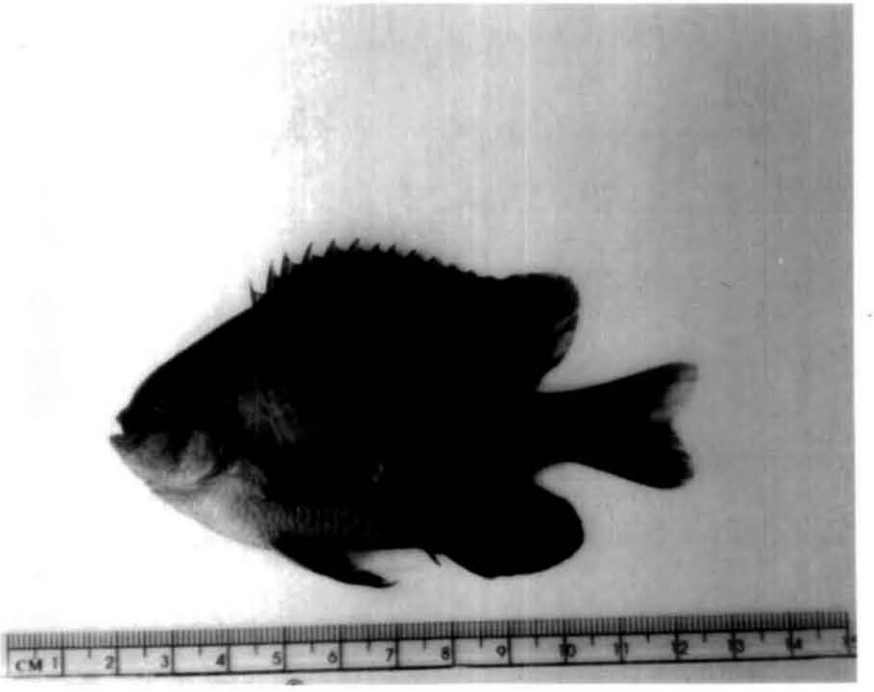
Number of specimen examined : 2
Size range of specimen : 88 and 92 mm (SL)
Collection site : Vizhinjam

Plate 7

a. *Dascyllus trimaculatus*



b. *Neoglyphydodon bonang*



7. Genus *Neopomacentrus* Allen, 1975

Body elongate. Middle rays of soft dorsal fin and anal fin and outer rays of caudal fin produced into long filaments. Sub orbital margin hidden by scales or exposed and smooth in some species. Moderately aggressive in aquaria.

1. *Neopomacentrus cyanomos* (Bleeker) 1856 - Regal demoiselle

Plate : 8a

Pomacentrus cyanomos Bleeker 1856
Pomacentrus leucosphyrus Fowler 1904
Pomacentrus prateri Fowler 1928

Body dark brown. Posterior part of dorsal fin and middle area of caudal fin creamy white to yellow. Outer margins of caudal fin distinctly dark. Blue spots present on dorsal and ventral parts of body. A dark blue blotch behind the opercle at the origin of lateral line and other at the upper part of pectoral fin base. Sub orbital margin hidden by scales. Body depth 2.2 – 2.3 (SL), head length 3.5 – 3.6 (SL) dorsal fin base 1.6 (SL). Eye diameter 2.8 – 3.2 (HL), inter orbital width 2.8 – 3.2 (HL), snout length 5.3 – 7.0 (HL), anal fin base 1.1 – 1.2 (HL) and caudal peduncle depth 1.8 (HL)..

Number of specimens examined : 18
Size range of specimen : 42 – 72 mm (SL)
Collection site : Vizhinjam.

Resembles *Neopomacentrus filamentosus* in colour pattern, but the sub orbital margin exposed in this species.

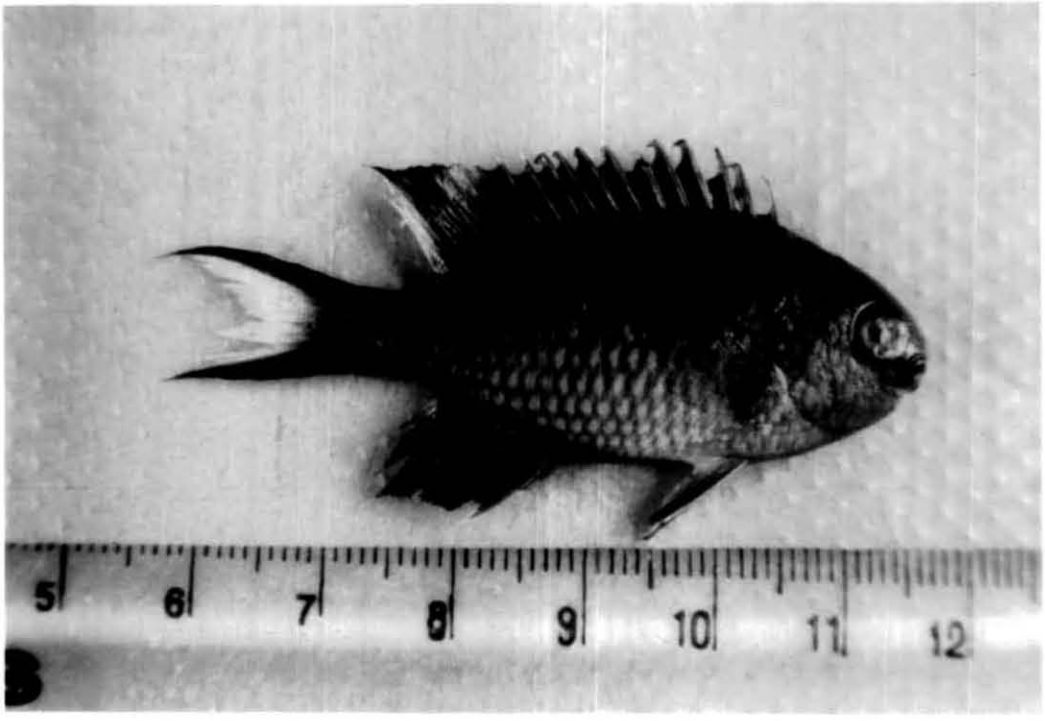
2. *Neopomacentrus taeniurus* (Bleeker) 1856 - Freshwater demoiselle

Plate : 8b

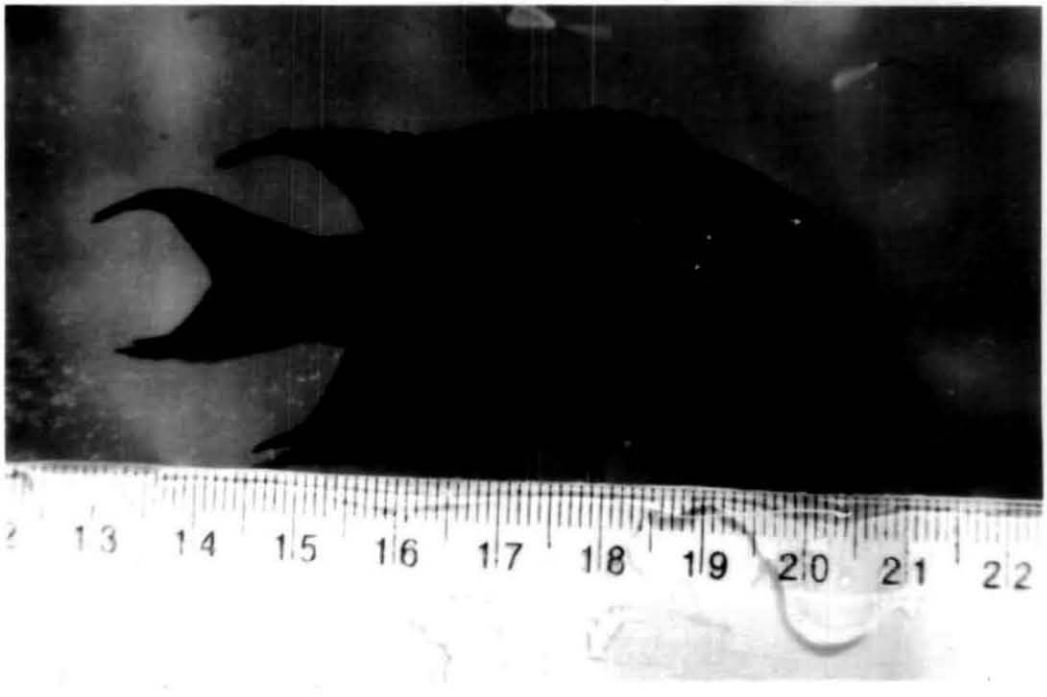
Pomacentrus taeniurus Bleeker 1856
Glyphydodon cochinensis Day 1865
Pomacentrus inhacae Smith 1955

Plate 8

a. *Neopomacentrus cyanomos*



b. *Neopomacentrus taeniurus*



Light grey to brown in colour. Posterior part of soft dorsal and middle part of caudal fin light grey. Outer caudal fin margins darker than inner area. Distal portion of caudal fin rays black. Dark blotch present at the origin of lateral line and pectoral fins. Sub orbital margins hidden by scales. Body depth 2.2 – 2.3 (SL), head length 3.1 - 3.2 (SL) dorsal fin base 1.5 – 1.7 (SL). Eye diameter 3.3 - 3.8 (HL), inter orbital width 2.4 – 2.6 (HL), snout length 3.8 – 4.3 (HL), anal fin base 1.1 – 1.2 (HL) and caudal peduncle depth 1.6 – 1.7 (HL). .

Number of specimens examined : 6
Size range of specimens : 40 – 54 mm (SL)
Collection site : Vizhinjam.

3. *Neopomacentrus nemurus* (Bleeker) 1857 - Coral demoiselle

Plate : 9a

Glyphisodon nemurus Bleeker 1857

Grey coloured dorsally and light ventrally. Posterior part of soft dorsal fin and anal fin yellow. Distal part of caudal peduncle and caudal fin yellow without dark margins. Dark blue blotch present at the origin of lateral line and origin of pectoral fins. Body depth 2.4 – 2.6 (SL), head length 3.7 – 3.8 (SL) dorsal fin base 1.8 (SL). Eye diameter 3.0 – 3.3 (HL), inter orbital width 2.4 – 2.7 (HL), snout length 4.3 – 6.0 (HL), anal fin base 1.2 – 1.3 (HL) and caudal peduncle depth 1.6 – 1.8 (HL).

Number of specimens examined : 8
Size range of specimens : 46 – 63mm (SL)
Collection site : Vizhinjam.

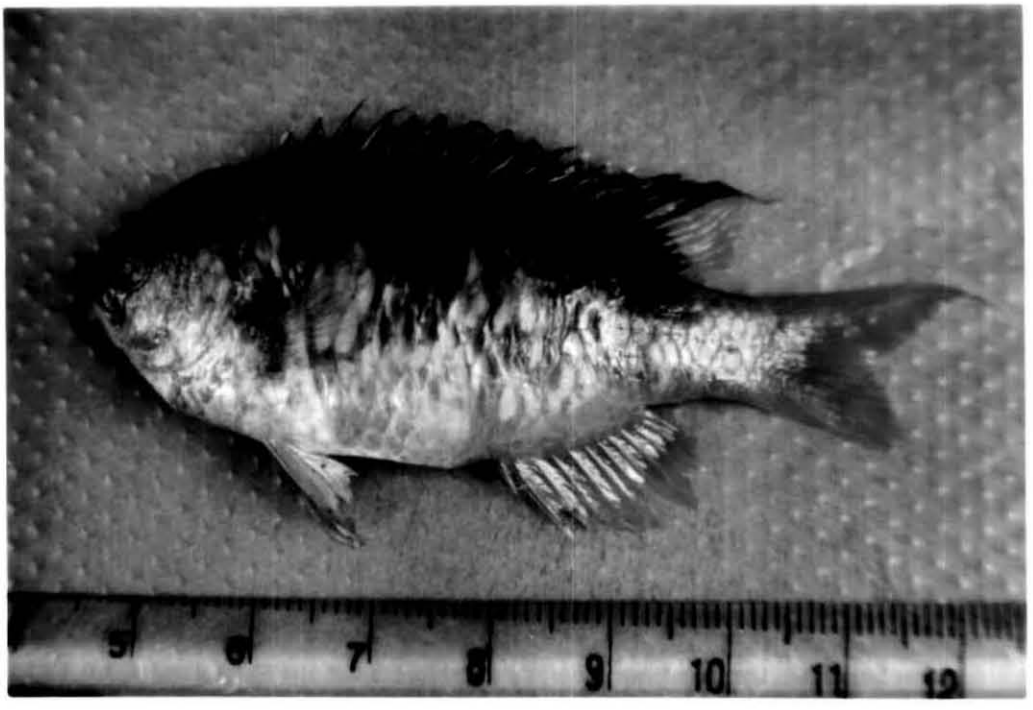
4. *Neopomacentrus sindensis* (Day) 1873 - Arabian demoiselle

Plate : 9b

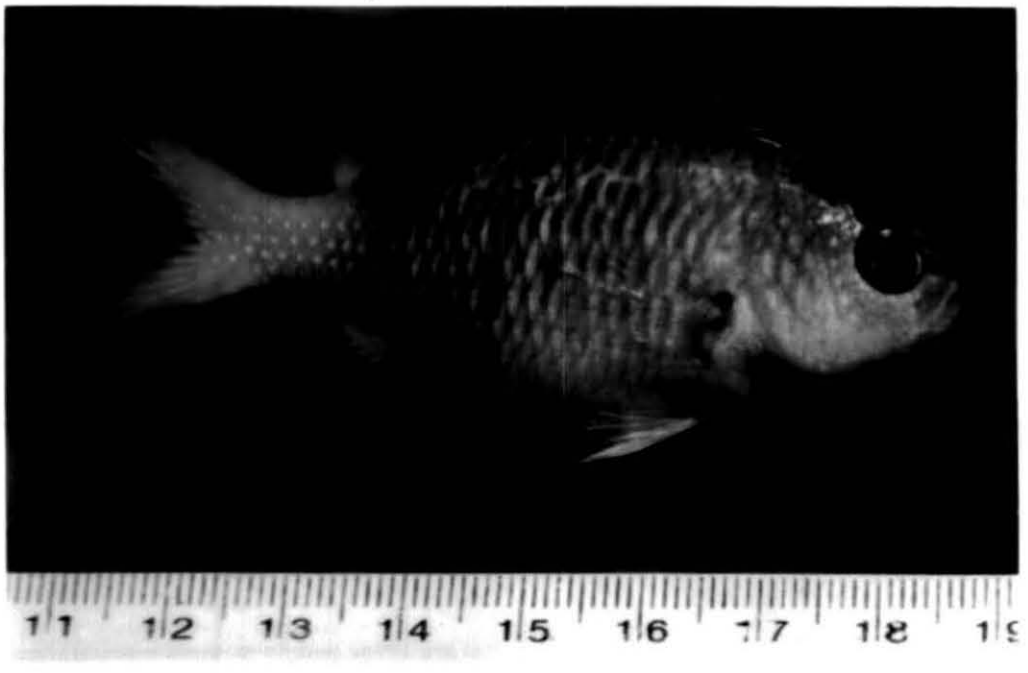
Glyphisodon sindensis Day 1873

Plate 9

a. *Neopomacentrus nemurus*



b. *Neopomacentrus sindensis*



Light to dark brown uniformly. Posterior part of soft dorsal fin yellow while the anal fin was not yellow. Posterior part of caudal peduncle and caudal fin yellow without dark margins. Dark blotch present at the base of pectoral fin. Depth 2.2 – 2.3 (SL), head length 2.9 – 3.0 (SL) and dorsal fin base 1.7 – 1.8 (SL). Eye diameter 3.8 – 4.0 (HL), inter orbital width 3.3 – 3.5 (HL), snout length 3.5 – 3.9 (HL), anal fin base 1.3 – 1.4 (HL) and caudal peduncle depth 2.5 – 2.7 (HL).

Number of specimen examined : 11
Size range of specimens : 24 – 84mm (SL)
Collection site : Vizhinjam.

8. Genus *Plectroglyphydodon* Fowler and Ball, 1924

Body relatively elongate, margin of pre orbital and sub orbital smooth, teeth uniserial, notch between pre orbital and sub orbital absent, sub orbital scaled. Members of this genus were rare in collections in the present study.

1. *Plectroglyphydodon lacrymatus* (Quoy and Gaimard) 1824 - Jewel damsel

Plate : 10a

Glyphisodon lacrymatus Quoy and Gaimard 1824
Glyphisodon nivosus Hombron and Jacquinot 1853
Glyphidodon florulentus Günther 1862

Body uniform brown in colour with many metallic blue spots on the dorsal half of the body above the lateral line. Caudal fin, posterior part of soft dorsal and anal fin pale. Body depth 2.0 (SL), head length 3.3 (SL) dorsal fin base 1.5 (SL). Eye diameter 3.2 (HL), inter orbital width 2.7 (HL), snout length 4.8 (HL), anal fin base 1.2 (HL) and caudal peduncle depth 1.9 (HL).

Number of specimen examined : 1
Size of specimen : 63 mm (SL)
Collection site : Vizhinjam.

2. *Plectroglyphydodon leucozonus* (Bleeker) 1859 - White band damsel

Plate : 10b

Glyphisodon leucozona Bleeker 1859
Glyphidodon cingulum klunzinger 1871
Abudefduf corneyi Jordan and Dickerson 1908
Abudefduf atrapinna Seale 1935
Chrysiptera yamashinai Okada and Ikeda 1937
Abudefduf melanozonatus Aoagi 1951

Body dark brown in colour with a white vertical bar originating from the level of sixth to ninth dorsal spine. The posterior part of the caudal fin and anal fin yellowish. Specimens with such characters are included in the sub species *Plectroglyphydodon leucozonus cingulum*. Body depth 2.2 (SL), head length 3.45 (SL) dorsal fin base 1.7 (SL). Eye diameter 3.6 (HL), inter orbital width 2.8 (HL), snout length 5.0 (HL), anal fin base 1.4 (HL) and caudal peduncle depth 1.9 (HL).

Number of specimen examined : 1
Size of specimen : 87 mm (SL)
Collection site : Vizhinjam.

9. Genus *Pomacentrus* Lacepede, 1802

Body elongate or moderately deep. Pre opercle and sub orbital serrate. A notch present between pre orbital and sub orbital. Sub orbital scale less. Teeth biserial. Generally aggressive in nature and some species are not advisable for community aquaria.

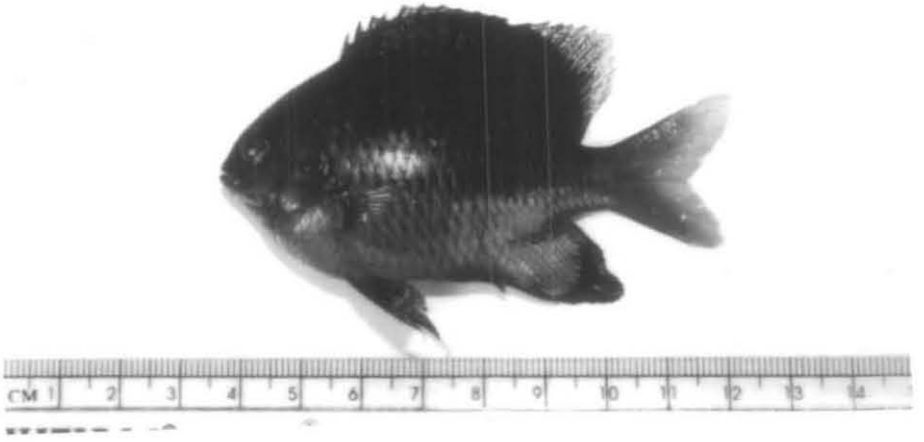
1. *Pomacentrus adelus* Allen 1991 - Obscure damsel

Plate : 11a

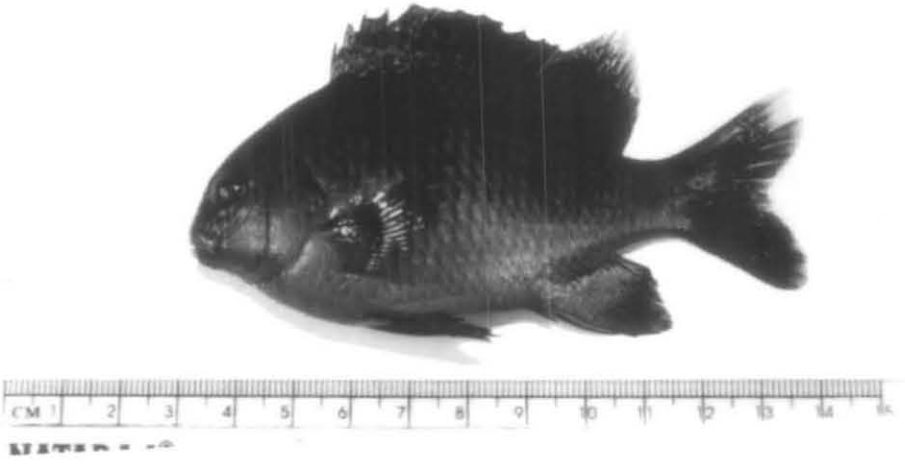
Medium to dark brown uniformly. A dark spot present at the origin of the of lateral line and upper part of pectoral fin base. Another dark spot present at the distal part of soft dorsal fin. Notch between pre opercle and sub

Plate 10

a. *Plectroglyphydodon lacrymatus*



b. *Plectroglyphydodon leucozonus*



opercle prominent. Body depth 1.9 – 2.0 (SL), head length 3.2 – 3.5 (SL), dorsal fin base 1.9 – 2.1 (SL). eye diameter 3.8 – 4.0 (HL), inter orbital width 2.1 – 2.2 (HL), snout length 3.2 – 3.3 (HL), anal fin base 2.0 – 2.1 (HL) and caudal peduncle depth 2.0 – 2.1 (HL).

Number of specimen examined : 2
Size range of specimens : 64mm and 66mm (SL)
Collection site : Vizhinjam.

Colouration is similar to *P. proteus* but the latter have 14 dorsal spines.

2. *Pomacentrus caeruleus* Quoy and Gaimard 1835 - Caerulean damsel

Plate : 11b

Pomacentrus pulcherrimus Smith 1960

Body metallic to dark blue in colour. Distal part of caudal peduncle and caudal fin usually yellow. Pectoral and pelvic fins yellowish. Distal margins of caudal fin sometimes dark. Yellow colour not present on distal parts of anal fin, or ventral parts of the body. Dark blue spot present at he origin of lateral line and upper edge of pectoral fin base. Notch between pre orbital and sub orbital weak. Body depth 2.6 – 2.7 (SL), head length 3.6 – 3.9 (SL) dorsal fin base 1.6 – 1.7 (SL). Eye diameter 3.5 – 4.0 (HL), inter orbital width 3.5 – 4.0 (HL), snout length 3.2 – 4.5 (HL), anal fin base 1.0 – 1.3 (HL) and caudal peduncle depth 2.3 – 2.7 (HL).

Number of specimen examined : 6
Size range of specimens : 42 – 66mm (SL)
Collection site : Vizhinjam.

Plate 11

a. *Pomacentrus adelus*

b. *Pomacentrus caeruleus*

Allen (1991) described the distinguishing features in the blue damsel complex. Comparable colour patterns have been described for *Pomacentrus coelestis* and *P. similis*. *P. coelestis* differs in having yellowish ventral region. Darker caudal margin and anal fin have been noticed for *P. similis*. But the darker nature of caudal fin is not a common feature in the present collection. Also the dusky and pale colouration of caudal fin vary in the same individual at different times in aquaria. The metallic blue colour is not present in *P. similis*.

3. *Pomacentrus pavo* (Bloch) 1787 - Peacock damsel , Blue damsel

Plate : 12a

Chaetodon pavo Bloch 1787
Holocentrus diacanthus Lacepede 1802
Pomacentrus pavoninus Bleeker 1853
Pomacentrus polynema Bleeker 1853
Pomacentrus furcatus Thiolliere 1857
Pomacentrus notatus De Vis 1883
Pomacentrus suvaroennsis Stead 1907
Pomacentrus caudovittatus Schmidt 1930
Pomacentrus hainanensis Wang 1941

Bluish green colour. Distal part of soft dorsal, anal and caudal fin pale. Head length 3.0 – 3.6 (SL), Body depth 2.4 – 2.5 (SL), dorsal fin base 1.6 – 1.8 (SL). Eye diameter 3.0 – 3.5 (HL), inter orbital width 3.5 – 4.0 (HL), snout length 4.5 – 4.7(HL), anal fin base 1.0 – 1.3 and caudal peduncle depth 1.0 – 1.5 (HL).

Number of specimens examined :- 3
Size range of specimens :- 27 – 51 mm (SL)
Collection site : Minicoy, Vizhinjam.

4. *Pomacentrus proteus* Allen 1991

Plate : 12b

Dark brown in colour throughout the body. Pectoral fin, posterior anal fin, dorsal fin and caudal fin pale. Black spot present at lateral line origin and pectoral fin base. Dark spot present at distal part of soft dorsal. Prominent notch between pre orbital and sub orbital. Body depth 2.0 – 2.2 (SL), head length 3.4 – 3.5 (SL) and dorsal fin base 1.6 – 1.7 (SL). Eye diameter 3.5 – 3.8 (HL), inter orbital width 2.3 – 2.6 (HL), snout length 3.2 – 4.5 (HL), anal fin base 1.0 – 1.1 (HL) and caudal peduncle depth 2.0 – 2.3 (HL).

Number of specimen examined : 5
Size range of specimen : 63 – 79 mm (SL)
Collection site : Vizhinjam.

Plate 12

a. *Pomacentrus pavo*

b. *Pomacentrus proteus*

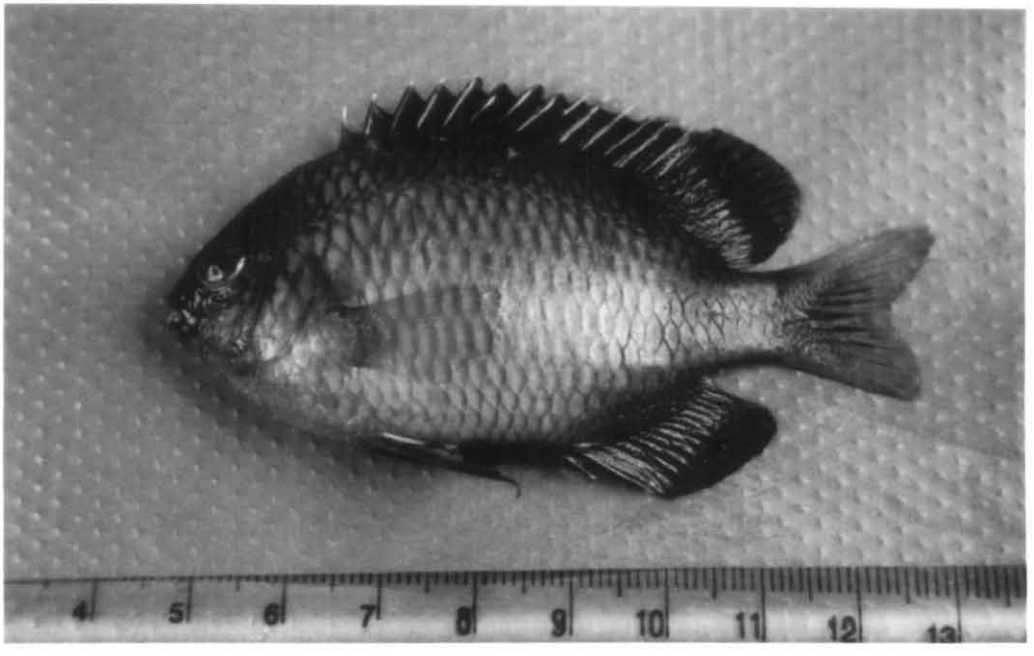


Table 1. Meristic counts of pomacentrids collected

Species	Lateral line scales	Transverse scale rows	Spine counts of fins		
			Dorsal	Anal	Pectoral
Abudefduf					
A. bengalensis	21 - 22	28 - 29	XIII, 12-13	II, 12-14	16 - 17
A.notatus	21	29	XIII, 13	II, 13	16
A.septemfasciatus	20	28	XIII, 14	II, 13	16
A. sordidus	19	28 - 29	XIII, 16	II, 15	16 - 17
A. vaigiensis	20 - 22	28 - 29	XIII, 12-14	II, 11-12	16 - 17
Amphiprion					
A. sebae	38 - 40	55 - 61	X-XI, 14-16	II, 13 - 16	14 - 17
Chromis					
C. viridis	16	27 - 28	XII, 10 - 11	II, 11	16
Chrysiptera					
C. biocellata	15	29	XIII,13	II,13	15
C. rollandi	18	27	XII, 14	II, 12	17
C. unimaculata	18 - 19	28 - 29	XIII, 12-13	II, 12-13	16 - 17
Dascyllus					
D. aruanus	17 - 18	26 - 28	XII, 12-13	II, 12	15 - 16
D. carneus	18	27 - 28	XII, 13-14	II, 12	17
D. trimaculatus	18	28 - 29	XII, 13 - 14	II, 12	15 - 16

Neoglyphydodon					
N. bonang	19	28 - 29	XIII, 15-16	II, 13	17 - 18
Neopomacentrus					
N. cyanomos	16 - 17	29 - 30	XIII, 10-11	II, 9 -10	15 - 16
N. taeniurus	17 - 18	29	XIII, 12	II, 12-13	16 - 17
N. nemurus	16 - 17	29	XIII, 10	II, 9 -10	15 - 16
N. sindensis	16 - 17	28 - 30	XIII, 10-11	II, 10 -11	16 - 18
Plectroglyphydodon					
P. lacrymatus	19	29	XII, 14	II, 12	15
P. leucozonus	20	29	XII, 14	II, 12	16
Pomacentrus					
P. adelus	19	27 - 29	XIII, 12-13	II, 12-13	16 - 17
P. caeruleus	17 - 18	27 - 28	XII-XIII, 15	II, 15	15
P. pavo	16	29	XII, 11	II,12	14
P. proteus	18	28 - 29	XIV, 13	II, 13 - 14	16 - 17

3. 4. Discussion

There are about 320 valid species belonging to 28 genera under the family pomacentridae. Of these, about 268 species have been reported from the Indo Pacific region which come to around 84 % of the world's total (Allen, 1991). The pomacentrid population in this region has been subjected to extensive taxonomic studies (Beaufort,1940; Woods and Schulz, 1960; Allen, 1972: Randall and Allen, 1977; Allen and Randall, 1980; Allen and Woods, 1980; Allen and Emery , 1985). The generic and species status of many species has changed in the recent revisions. The most recent compilation on the systematics of this family as a whole is by Allen (1991), and hence followed for describing the generic and species status in the present study.

In spite of the plentiful distribution of coral reef areas and associated fish fauna in India, much attention has not been given to the studies on reef fishes, and the pomacentrids are no exception. Reliable and updated works on the distribution and systematics of this family are scanty from our waters. Jones and Kumaran (1980) gave a systematic account of the fishes of Laccadive Archipelago and described 35 pomacentrid species. Kuthalingam *et al.* (1979) described two damsel fishes from Vizhinjam. Comprehensive survey of our reef areas is needed to record and describe all the species of pomacentrids.

In the present study, samples were collected mainly from the rocky areas off Vizhinjam in the southwest coast of India. Eighteen species belonging to eight genera were collected from this region. The other areas of collection were Minicoy Island in Lakshadweep and Rameswaram. But extensive surveys were not made in these areas. Out of the 24 species described in the present study, six species are new records from the area – *Chrysiptera rollandi,*

Neoglyphydodon bonang, Neopomacentrus nemurus, Neopomacentrus sindensis, Pomacentrus proteus and *Pomacentrus adelus.* It is felt that the species obtained in the collection for this study represent only a fraction of the pomacentrids distributed in indian waters. Detailed exploratory and sampling of the coral reef areas of the country such as Lakshadweep, Andaman and Nicobar islands, Gulf of Mannar, Palk bay and Gulf of Kutch will bring to light many more species of pomacentrids.

The pomacentrids exhibit much variation especially in colour patterns depending on geographical areas and even within the same distributional area. The characters and colour patterns often overlap among species and also different colour variants of a species may closely resemble another species. This was exemplified by the variation noted in the present study in *Amphiprion sebae* and *Pomacentrus caeruleus.* Here the variations manifested even within individuals of the same brood. Hence the possibility of such colour variations must also be taken into account in the taxonomic studies of this diverse group.

4. REPRODUCTIVE BIOLOGY OF TWO SPECIES OF POMACENTRIDS WITH SPECIAL REFERENCE TO SEX REVERSAL

4.1. Introduction

Reproduction in reef fishes has always been a topic of interest to marine biologists the world over. Many reef fishes have adaptive mechanisms in reproduction to suit the peculiarities of the environment which they inhabit. These include territoriality, colonial nesting patterns, social control of breeding , hermaphroditism, sex reversal and parental care. Most of the pomacentrid fishes exhibit these features. Field observations and results from field set experiments have yielded valuable information on the above aspects of many species. Later more attention was paid to the studies on the reproductive biology and reproductive physiology.

Fricke and Fricke (1977) reported protandry in the anemone fishes *A. akallopisos* and *A. bicinctus*. They suggested the aggressive dominance by the high ranking fish in a colony as the reason for monogamy and protandry in these species. Shapiro (1984) described the social aspects of sex change in both directions. The different social interaction mechanisms causing sex change in different species and their adaptive significance to their environments was described by Ross (1990).

The detailed structural characteristics of the bisexual gonad of *Amphiprion frenatus* was described by Brusle-Sicard and Reinboth (1990). The ultrastructural changes occurring at different stages to the gonads of protandrous hermaphrodite *Amphiprion frenatus* were described by Brusle-

Sicard *et al.* (1994). The ultrastructure and distribution of steroidogenic cells in the gonads of *A. frenatus* was described by Nakamura *et al.* (1994). The gonadal structure of a protogynous hermaphrodite *Dascyllus reticulatus* was described by Schawarz and Smith (1990). There are reports on the hermaphroditism in other groups of fishes also (Debas *et al.*, 1989; Kokokiris *et al.*, 1999). Tzioumis and Kingsford (1999) reported the gonochoristic nature of a damselfish *Parma microlepis* .

In Indian waters, investigations on the reproductive biology and ecology have been carried out only on a few species of pomacentrids (Pillai *et al.* 1987a; 1987b; Pillai and Madanmohan, 1990; Madanmohan *et al.*, 1987). Murty (2002) described the resource characteristics and bilogy of ornamental fishes of Lakshadweep including pomacentrids. Vijayanand (1994) studied the reproductive biology of two species of pomacentrids. However, detailed studies on the maturity stages and sex change have not yet been done in Indian waters. In this context this study was taken up to investigate the different maturity stages and hermaphroditism of two species of pomacentrids, *Amphiprion sebae* and *Neopomacentrus cyanomos* .

4.2 Materials and Methods

4.2.1. Specimens

Clownfishes for the study were collected from Rameswaram in May 2001. A total of 175 specimens were collected . The sample contained individuals of all size groups starting from newly settled juveniles. The damselfish *N. cyanomos* was collected from Vizhinjam during November 1999 to May 2001. Samples were collected in all the months during the period. However, all the length groups could not be collected every month. A total of 327 specimens were collected during the period of study.

4.2.2 . Examination of the specimens

The standard length of all the specimens was taken to the nearest millimeter. The gonads were removed and were examined to determine the maturity stages. Size frequency data of males and females of both species were prepared. In case of the anemonefish all sub adults were taken as males and transforming individuals as females.

4.2.3. Determination of maturity

The maturity stages of ova were determined as described in Schwarz and Smith (1990). The female gonads of *N. cyanomos* were classified into three as described in Qasim (1971). They were (i) immature, (ii) maturing and (iii) ripening. Ovaries were considered immature when they contained stage I and stage II ova, maturing, when they contained stage III ova and ripening when stage IV and V ova were present.

4.2.4. Size frequency data of ova

For anemonefishes five fully mature ripening ovaries were selected for ova diameter measurements. In the case of *N. cyanomos* four ripening ovaries collected in November 1999 and three maturing ovaries collected in May 2000 were used for taking ova diameter measurements. A small portion of the ovary was taken and all the eggs in that area were measured using an ocular micrometer through a monocular microscope. Since the eggs of both the species were capsule shaped, lengths of eggs

were measured instead of diameter. Number of stage IV and stage V eggs present in the ovaries were counted using a stereo microscope.

4.2.5. Histology

Samples from each stage were taken for histological analysis. They were fixed in 5% buffered formalin for 24 hours and transferred to 70% isopropanol. They were embedded in paraffin wax and sectioned at 5-7 µm thickness and stained with haematoxylin and eosin.

4.3.Results

4.3.1. *Amphiprion sebae*

The size frequency distribution of males and females is given in Fig.1. The smaller size group upto 65 mm comprised exclusively of males - either functional or non functional. The size range of females was 65 - 95 mm standard length and all the individuals above 85 mm were females. Both males and females were present in the overlapping range of the two size frequency curves *ie*, 65 to 85 mm . The male to female ratio was 5:1 in the entire sample and 1.3 :1 in the overlaping range. This is a clear indicator of the protandrous nature of the species.

The ovadiameter frequency polygon of *A. sebae* is shown in Fig.2. There was a general pattern in all the ovaries examined in having three distinct peaks corresponding to three different size groups of eggs. Eggs of size 35 divisions were fully mature stage IV and V eggs and those in the

Fig. 1. Size frequency distribution of males and females of *A. sebae*

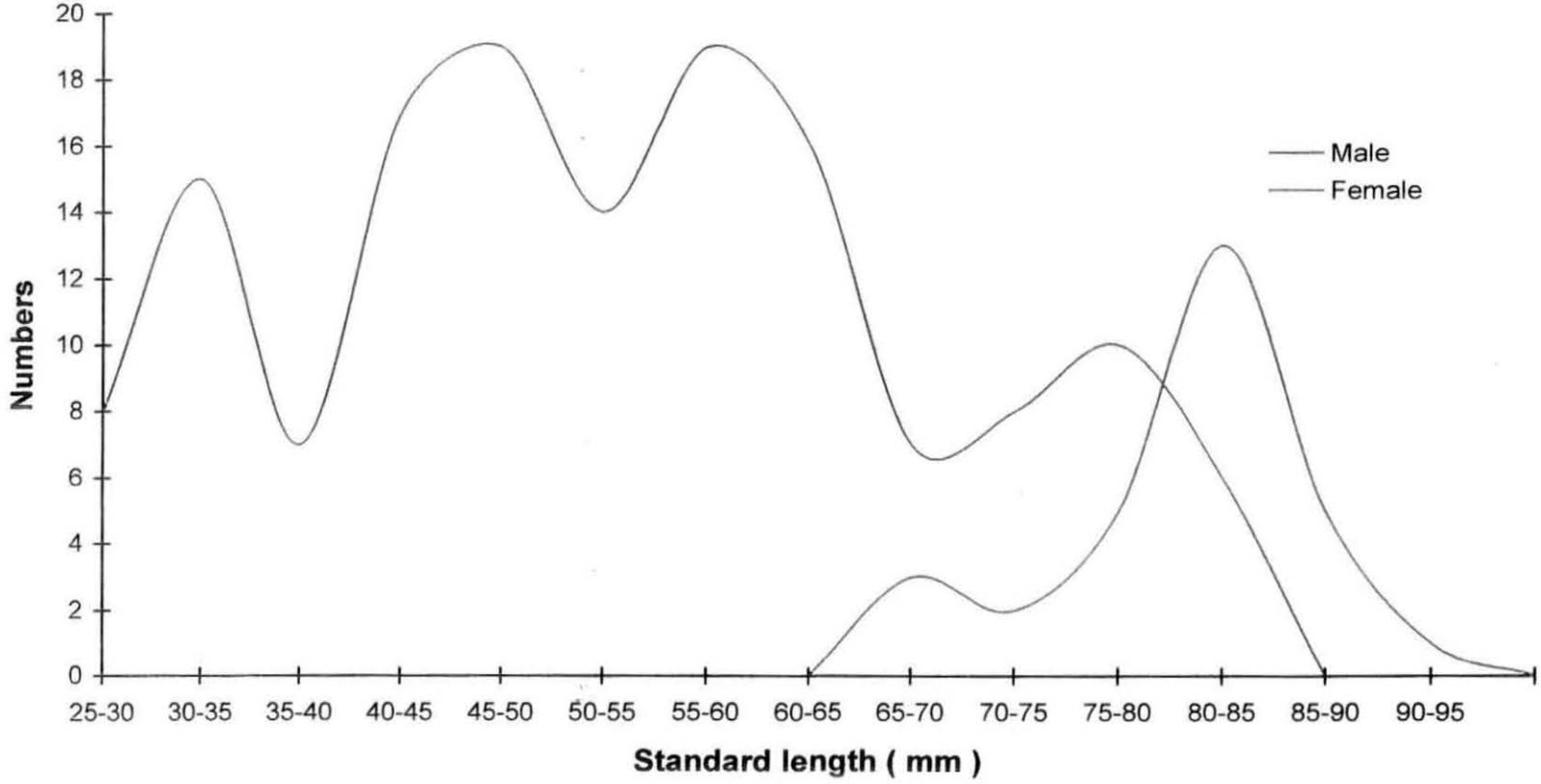
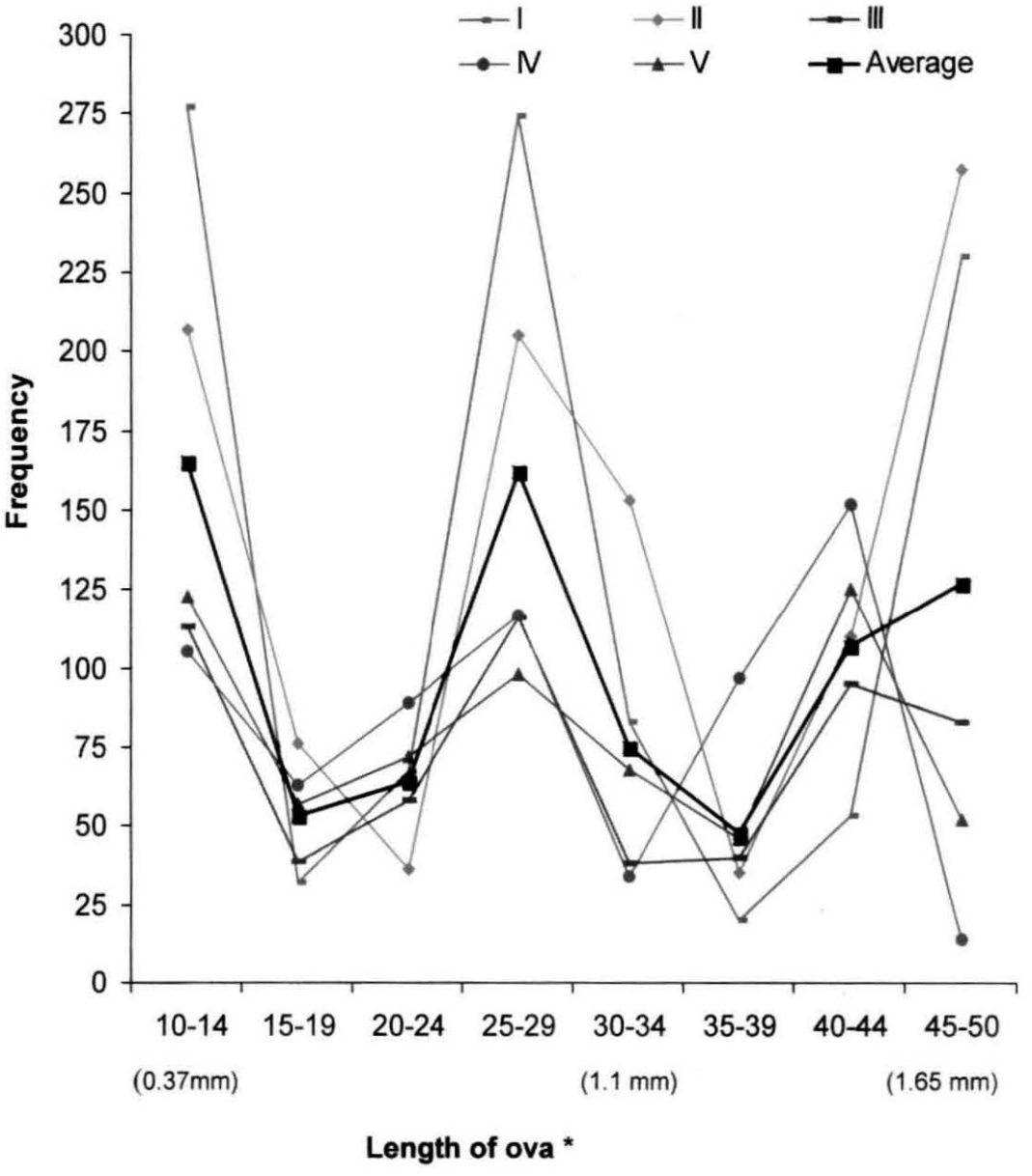


Fig. 2. Size frequency distribution of ova in fully mature ovaries of *A. sebae* collected in May 2001

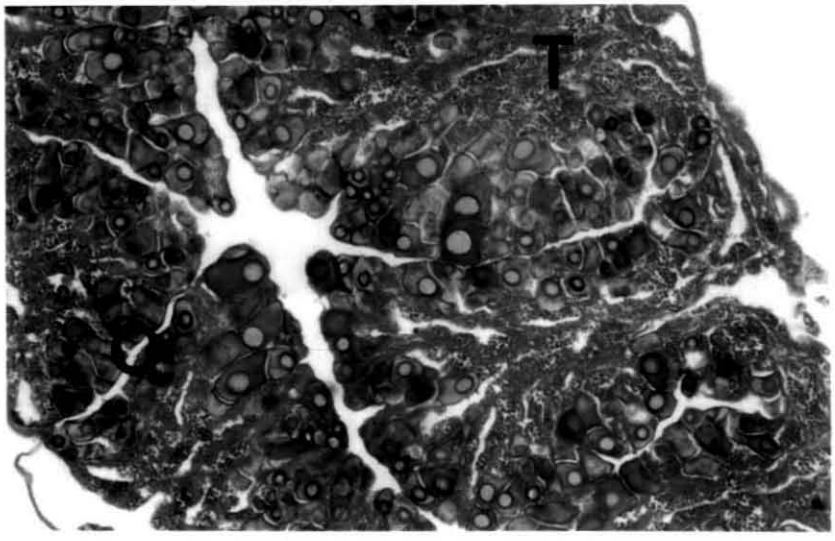


* The range is given in micrometer reading. The equivalent range in millimeter is given in parentheses

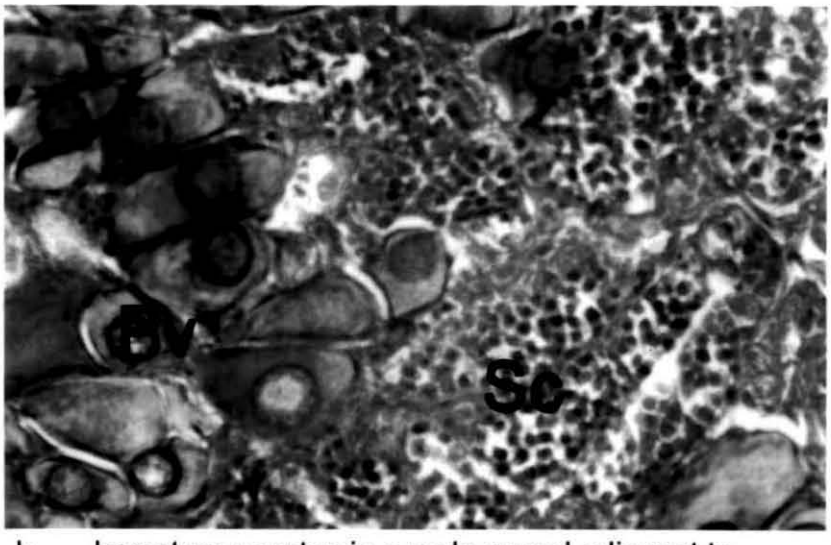
range of 20-35 divisions were maturing stage III eggs and the smaller group of eggs below 20 divisions were immature stage I and stage II oocytes. The average number of mature ova in the ripening ovaries in the sample was 1869.82 ± 673.20.

The histological analysis of the gonads of *A. sebae* provided conclusive evidence for the ambisexual nature of gonads and protandrous hermaphroditism. Cross section of a functional male gonad is shown in Plate1a. The gonad is an ovotestis with an inner ovarian part and outer testicular part and a lumen at the center. The ovarian part comprises of immature previtellogenic oocytes. Spermatogenic cysts and seminiferous tubules were visible in the testicular part. The testicular and ovarian part were contiguous without any connective tissue separating them (Plate 1b). In the non- functional male gonad (Plate 1c) the ovarian part of the ovotestis occupied more area compared to the testicular part. But the development of the spermatogenic cysts was not suppressed. The transforming gonad (Plate 2a) was more tubular in shape compared to the flattened shape of functional testis. Spermatogenic cysts were far less in number. It appeared more or less like an immature ovary with previtellogenic oocytes. The degenerating testicular tissues were distinguishable along with developing ovarian parts (Plate 2b). Fully transformed mature ovary is shown in Plate 2c. It maintained ripe ova with numerous oil and fat globules in the cytoplasm, maturing as well as immature oocytes. There were no signs of the presence of testicular tissues in mature ovaries. Therefore the male gonads were ovotestis whereas the female gonads were pure ovaries.

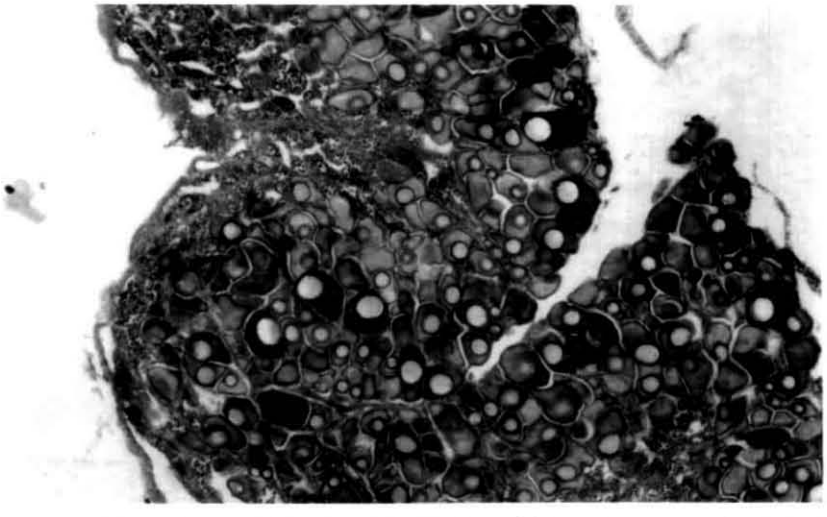
Plate 1.Histological sections of male gonads of *A. sebae*



a. Functional male gonad with inner ovarian and outer testicular parts
O - Ovarian Tissue T- Testicular Tissue

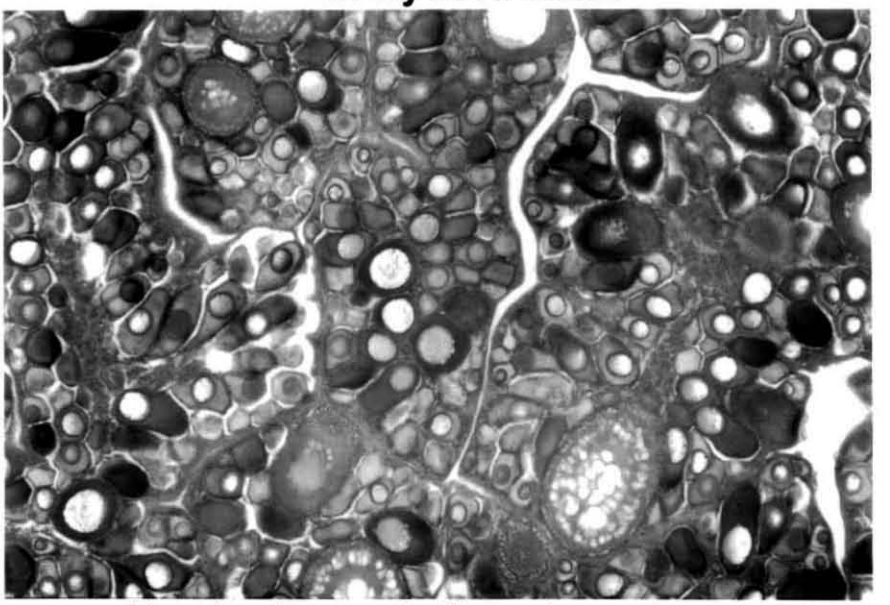


b. Immature oocytes in a male gonad adjacent to spermatogenic cysts which are numerous in number
Sc - Spermatogenic Cysts Pv - Previtllogenic Oocytes

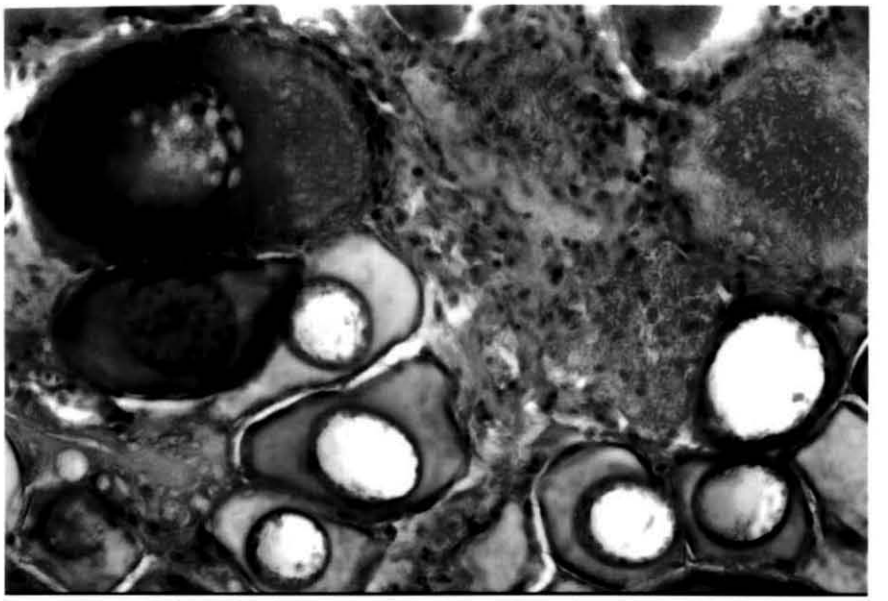


c. Gonad of a non functional male with greater ovarian area

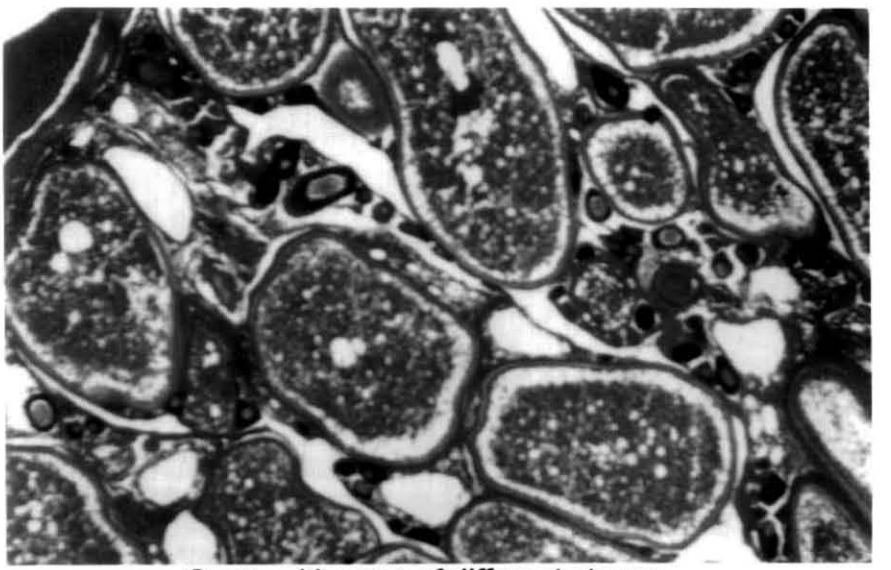
Plate 2. Section of a transforming gonad and a mature ovary of *A. sebae*



a. Transforming gonad with greater ovarian area



b. Developing oocytes in a transforming gonad contiguous to the spermatogenic cysts



c. Ovary with eggs of different stages

4.3.2.Neopomcentrus cyanomos

The size frequency distribution of males and females of *N. cyanomos* is shown in Fig. 3. The smaller size group up to 65 mm standard length were all females at different stages of maturity. All the individuals above 80 mm were functional males with well developed testis. The overlapping range of the two curves between 65 mm and 80 mm comprised of both males and females. Female to male ratio in the entire sample was 4.6 :1 and the same in the overlapping length range was 0.83 :1 . This points to the protogynous nature of the species.

Ripening ovaries predominated in the sample from July to February-March, but were fewer thereafter till June (Fig. 4). The maturing ovaries showed two distinct peaks corresponding to two major size groups of eggs (Fig. 5). The eggs that were longer than 19 divisions were maturing stage III oocytes and the smaller ones were immature eggs. Ripe eggs which were ready to be released were not found in the ovary. Ova diameter frequency polygon of ripening ovaries had three distinct peaks (Fig. 6). Immature eggs measured up to 12 divisions , maturing stage III eggs measured 16 – 21 divisions and ripe eggs which were ready to be released measured more than 22 divisions. The average number of ripe eggs in the ovaries were 2779.6 ± 835.5. The size at first maturity of females was estimated to be 57 mm standard length (Fig. 7).

Conclusive information on bisexuality of gonads could not be obtained from histological studies. Ovarian tissue remnants were not present in functional testis (Plate 3a) and also spermatogenic cysts were not found in mature ovaries (Plate 3b) as well as in immature ovaries (Plate 3c) . The ripening ovaries contained ripe eggs with numerous yolk globules in the cytoplasm as well as maturing and immature oocytes.

Fig.3. Size frequency distribution of males and females of *N. cyanomos*

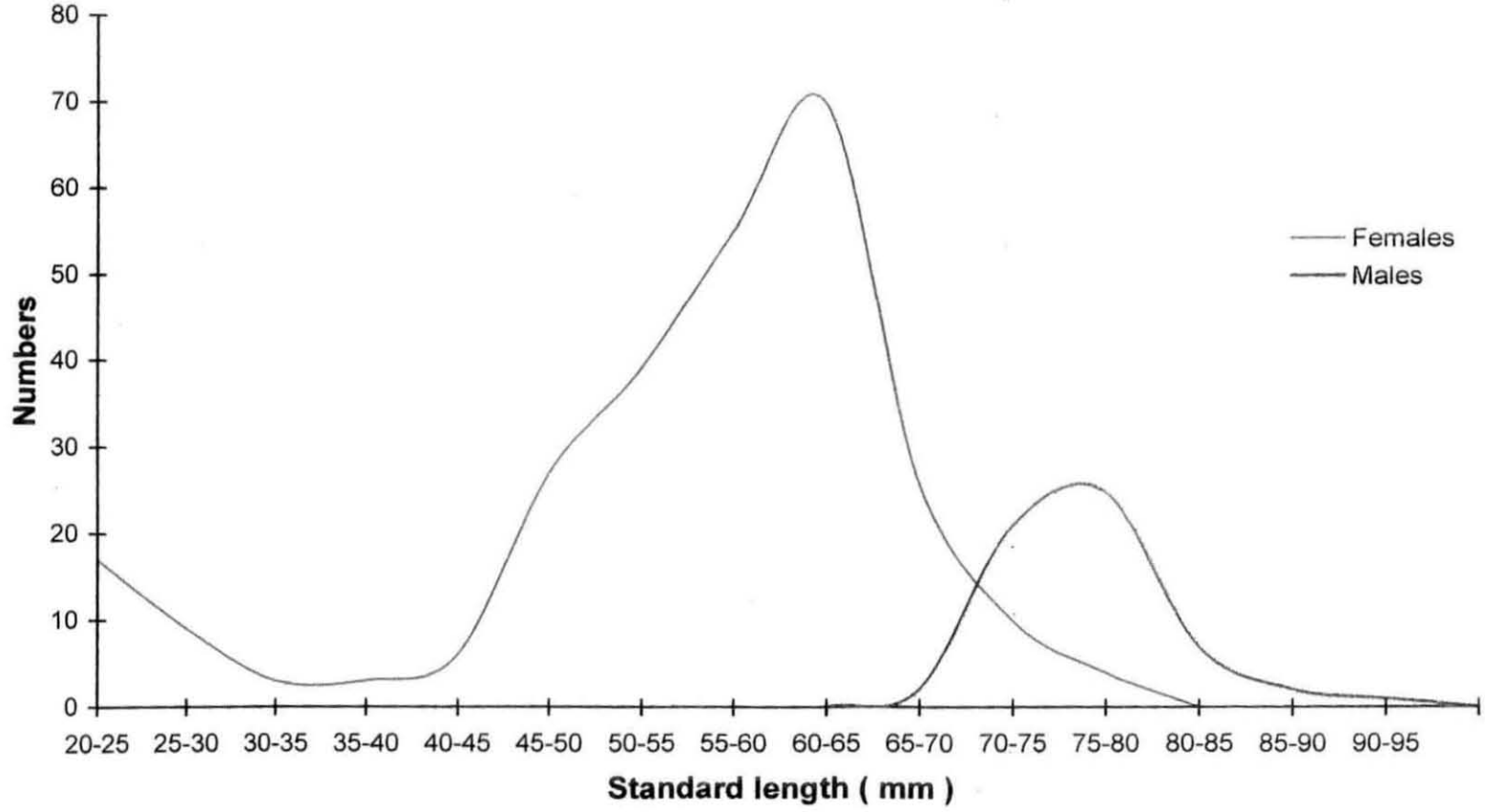


Fig. 4. Frequency of occurrence of different maturity stages of female *N. cyanomos* from November 1999 to May 2001

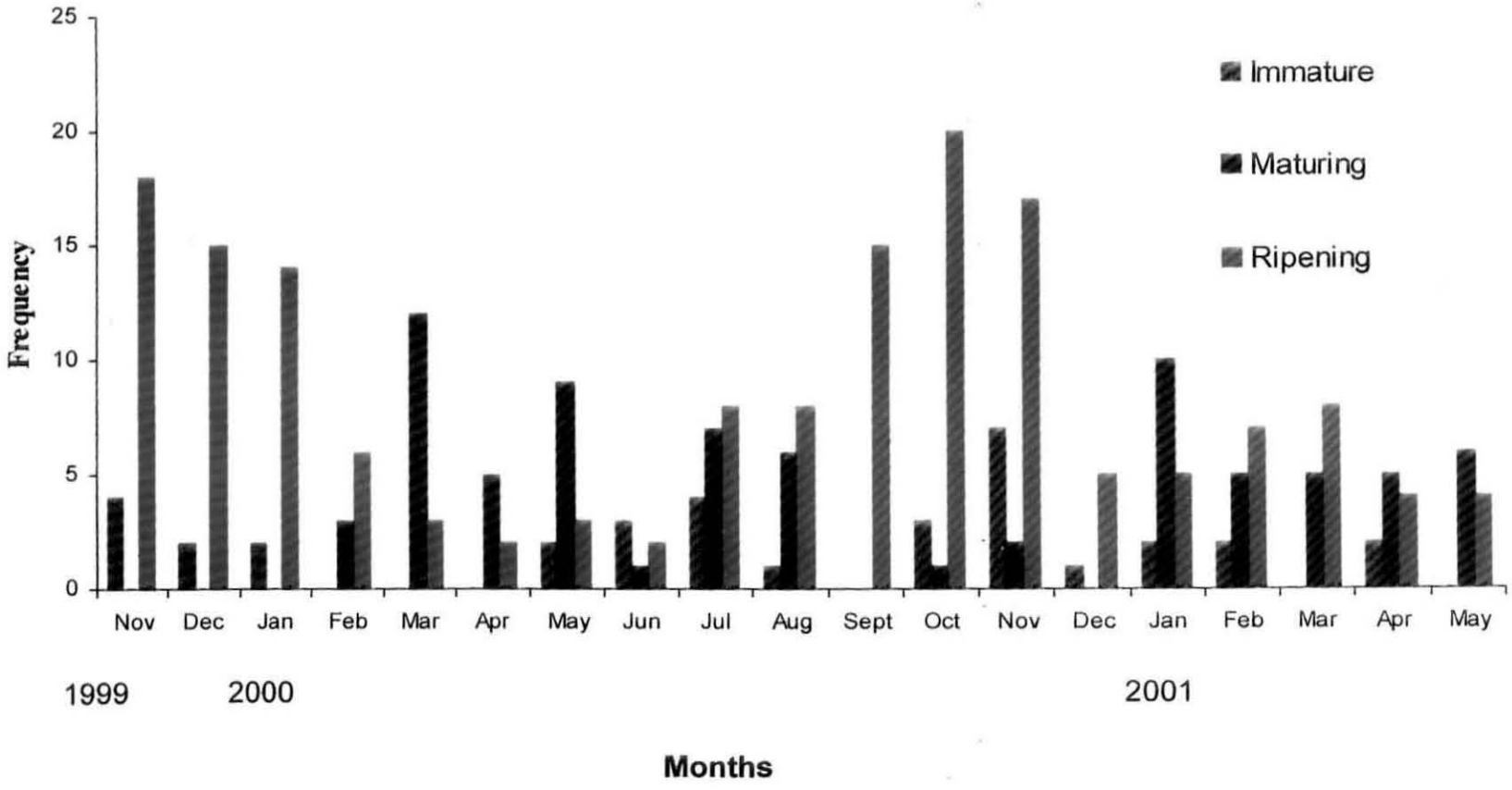
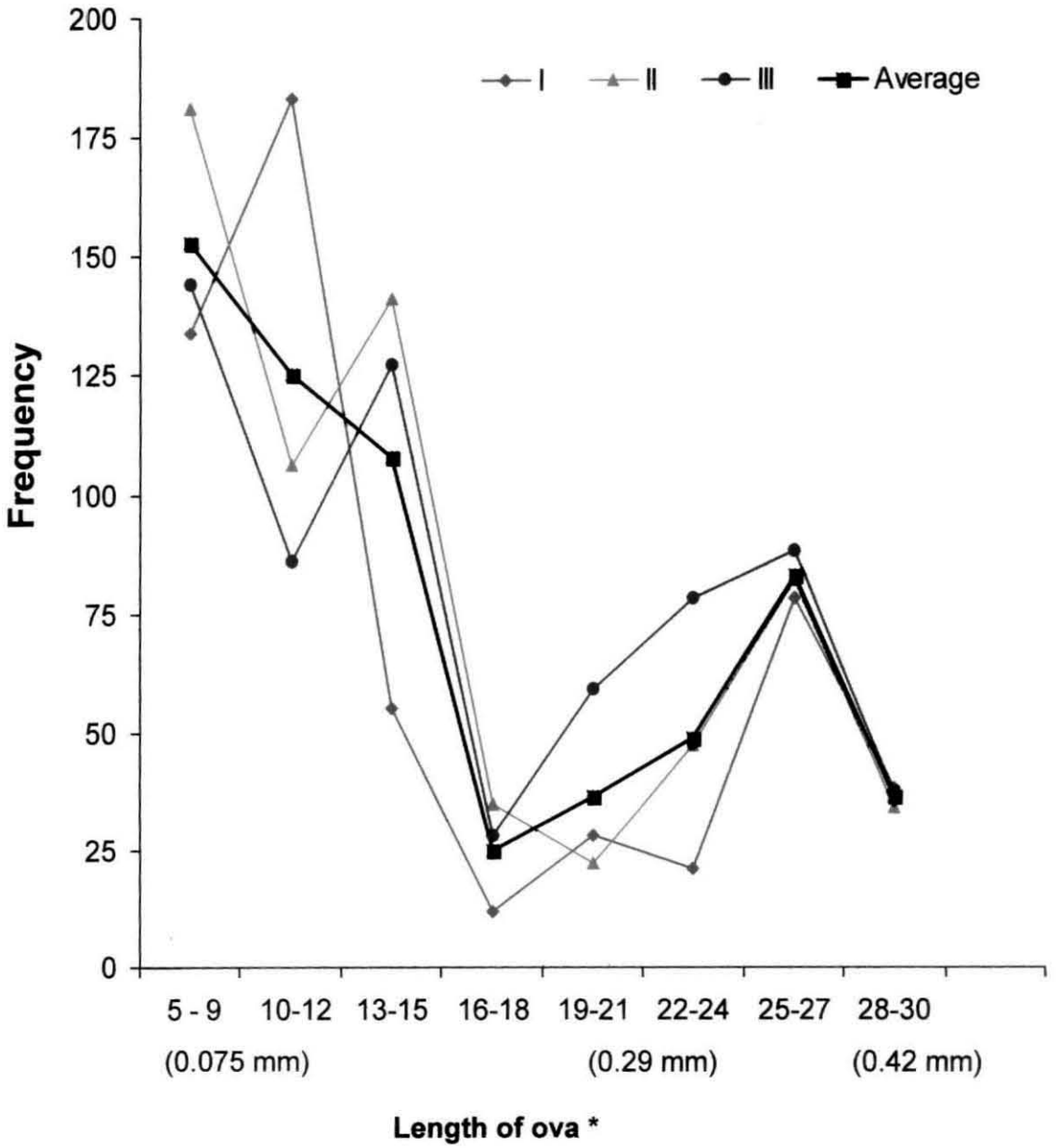
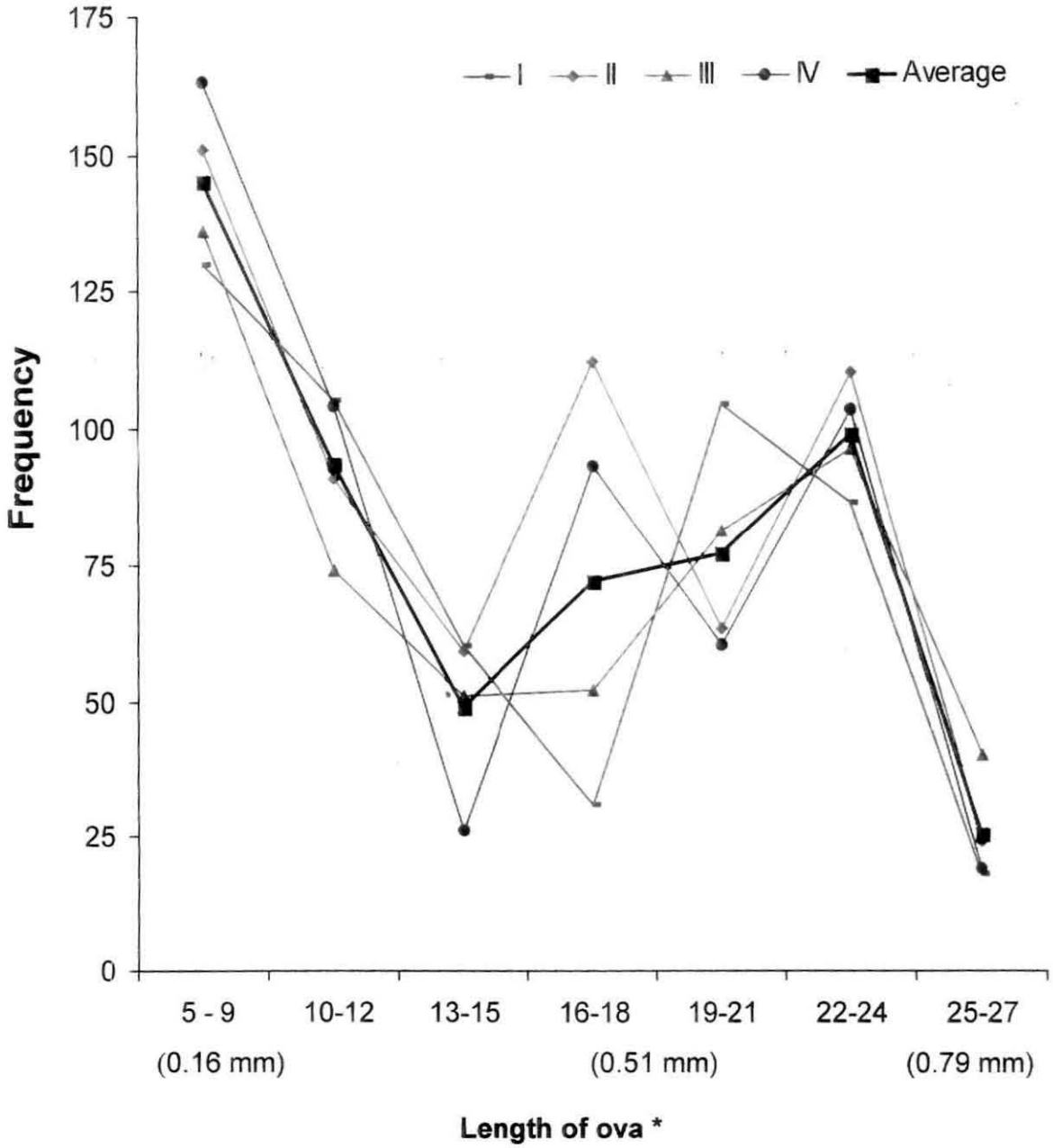


Fig. 5. Size frequency distribution of ova of mature ovaries of *N. cyanomos* collected in May 2000



* The range is given in micrometer reading. The equivalent range in millimeter is given in parentheses

Fig.6. Size frequency distribution of ova of mature ovaries of *N. cyanomos* collected in November 1999



* The range is given in micrometer reading. The equivalent range in millimeter is given in parentheses

Fig.7. Cumulative frequency of mature females for different size groups in *N. cyanomos*

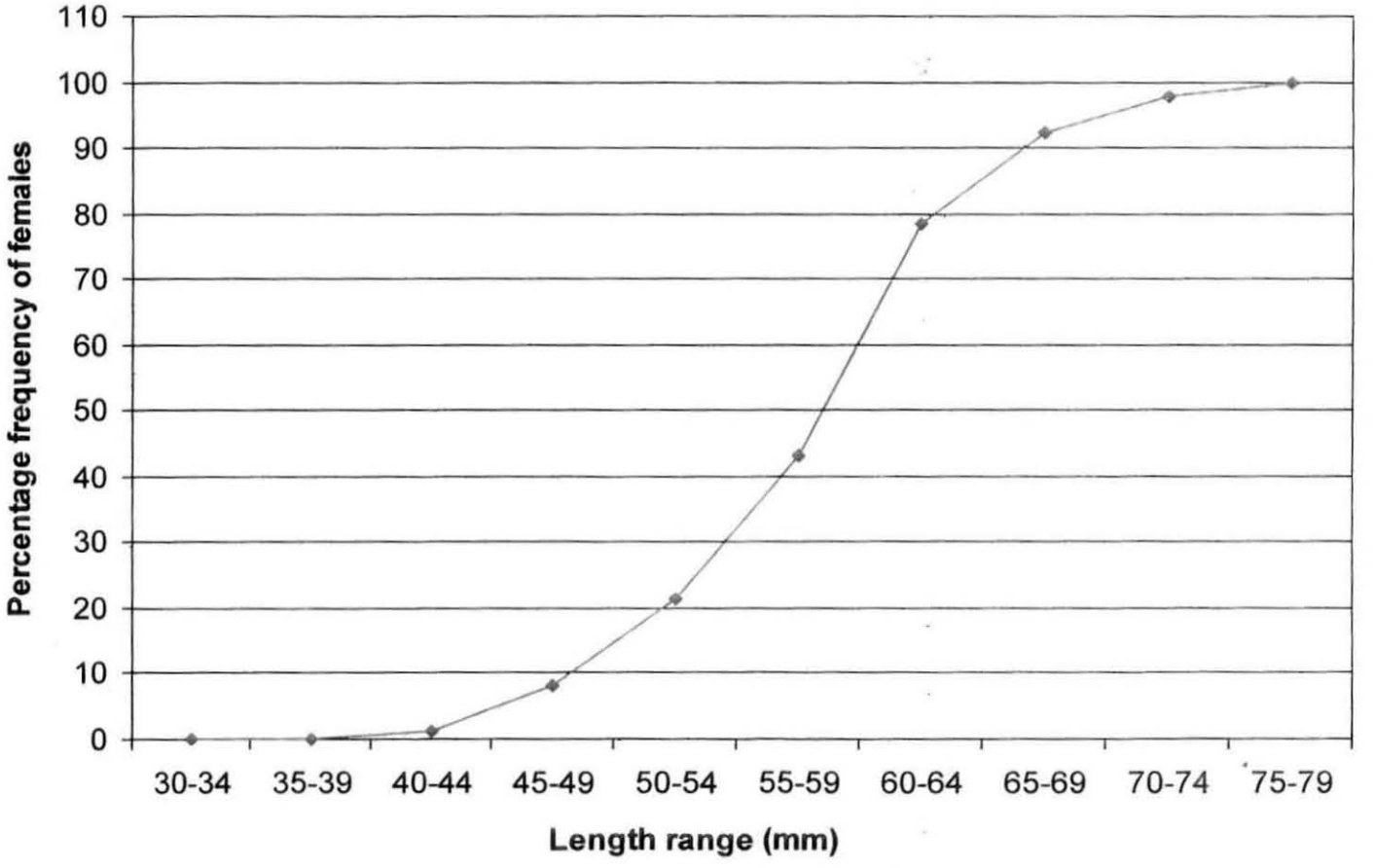
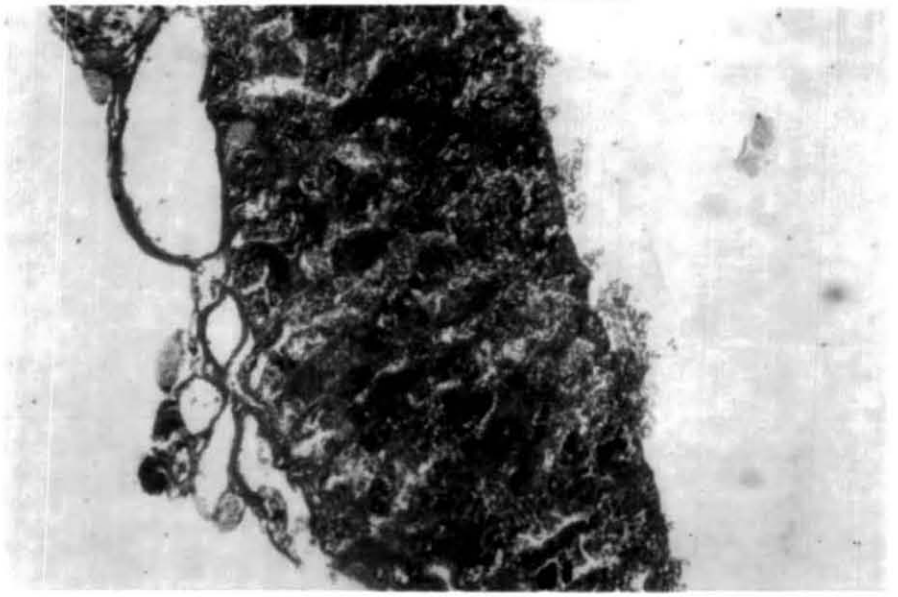
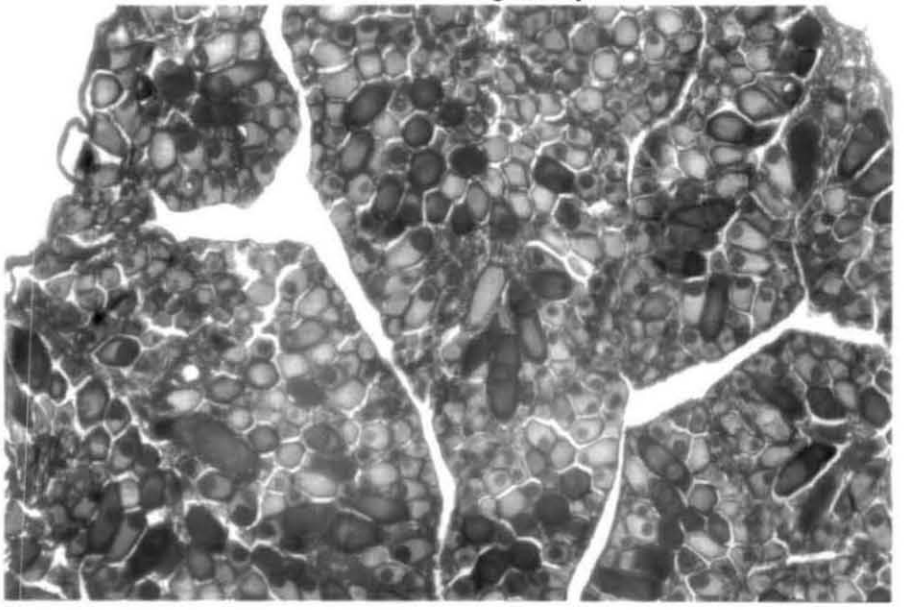


Plate 3

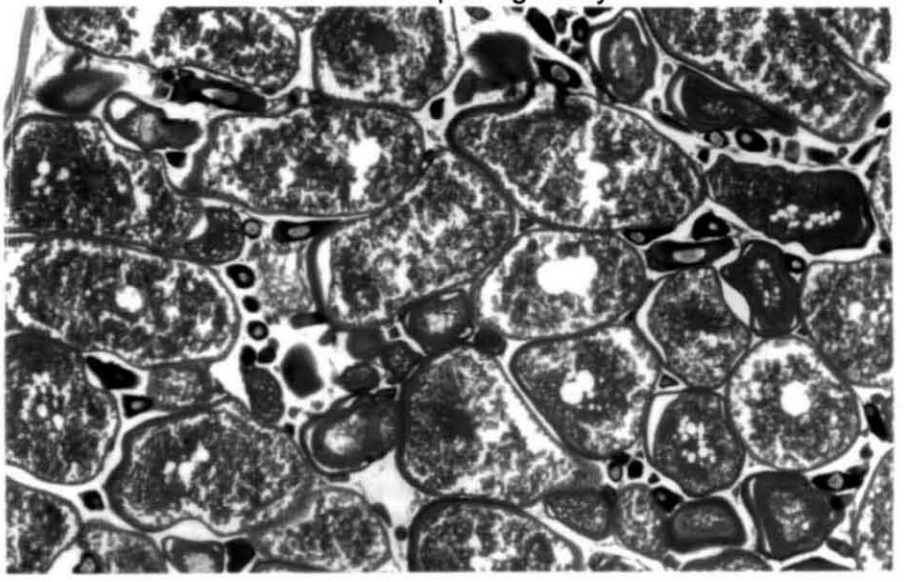
a. Section of a functional testis



b. Section a maturing ovary



c. Section of a ripening ovary



4. 4. Discussion

Family pomacentridae includes protogynous and protandrous hermaphrodites and gonochorists (Ross, 1990). Protandrous hermaphroditism has been reported from many species of anemone fishes and protogynous hermaphroditism is reported in some species of damselfishes. Size frequency distribution of both sexes of a population can be taken as one of the indicators to the direction and size of sexual transformation. In protandrous hermaphrodites all the individuals in the smaller size groups will be males , non functional or functional, and the largest size groups will be mostly females. In protogynous hermaphrodites all the individuals in the smaller size groups will be females and larger individuals will be mostly males. The early developmental stages of the terminal sex will be absent in lower size groups. The size frequency data of *A. sebae* is thus indicative of protandrous hermaphroditism and that of *N. cyanomos* of protogynous hermaphroditism.

Gonads of sub adults or γ individuals of anemonefishes which face suppression from both functional male and functional female look like immature ovotestis where the development of testicular tissue is suppressed (Brusle-Sicard, *et al.* 1994; Fricke and Fricke,1977). The subadult examined in the present study was not from a group containing a functional pair, but from a maturing group of juveniles. Hence the onset of testicular development in the present specimen can be attributed to this factor. Gonadal changes in juveniles of *A. frenatus* from a single brood without adult individuals was described by Brusle-Sicard *et al.* (1994) where the gonad of the largest individual exhibited ' abnormal spermatogenic activity ' and the gonad of the second largest fish exhibited active spermatogenic activity. The gonad of the third ranking fish was a typical immature ovotestis. Thus this sub adult in the study has started differentiating into a functional male.

Functional male gonad of *A. sebae* in the present study resembles to those described by Fricke and Fricke (1977), Brusle-Sicard and Reinboth (1990) and Brusle-Sicard *et al.* (1994). In this case the male and the female germ cells are contiguous without any connective tissue separating them and that condition is unique to this group (Brusle-Sicard and Reinboth, 1990). Sex reversal is not the best way for reproductive success for a male which lost its female partner as this may cause a time lag for subsequent spawning (Ochi and Yanagisava, 1987). Maintaining bisexual gonad may be an adaptation for minimising this time lag (Ross, 1990).

A transforming gonad is characterised by the degeneration of the male germ cells and associated sertoli cells. Macrophages with phagocytic vacuoles are also present in this stage (Brusle-Sicard *et al.* 1994). The time taken for transformation was about 10 weeks in *A. frenatus.* The testicular development of a juvenile was faster in the presence of a functional female than in the presence of a functional male (Brusle-Sicard *et al.*, 1994). The sex inversion is likely to be irreversible as there was no testicular tissue left in a mature ovary.

In monogamous fishes like anemonefishes the sex inversion must be quicker when it becomes necessary to transform because there will be no other female in the colony to take over the spawning. Moreover, the males have the risk of the colony being taken over by intruding larger fishes. But in the case of the damselfish *N. cyanomos* which is polygynous the need for quick transformation is very less and there will be usually more than one male in a colony .Mostly the males defend nesting sites and the females are attracted into it. Thus the loss of one male does not cause any immediate breeding failure. Therefore it is not necessary for the females to maintain ovotestis. The absence of distinct testicular tissues in ovaries at any stage can be attributed to this factor. But the size - frequency distribution clearly indicates protogyny in *N. cyanomos.* Asoh *et al.* (2001) reported the non

availability of gonads of *Dascyllus albisella* containing degenerating vitellogenic oocytes and proliferating spermatogenic tissue but gonads with degenerating cortical alveolus stage oocytes and developing spermatogenic tissues were present. They opined that sex reversal to male in *D. albisella* occur before final oocyte maturation and spawning as female.

Both the species under study were continuous breeders. This was observed directly by maintaining them in captivity. Also mature individuals of both sexes were collected almost round the year for *N. cyanomos*. Qasim (1971) suggested to classify ovaries of continuous breeders into three maturity stages. Spent ovaries were not observed for both the species. However, maturing ovaries were occasionally found in larger individuals of both species. This indicates that spawning and maturation of ova in the ovary are continuous processes and when ripe ova are released at a faster rate than the maturation, the ovaries lack ripe ova for a short duration till the maturing ova become ripe. This can lead to small pauses in spawning after prolonged spawning periods. Such phenomenon was noted for both the species in captive conditions. However, a cyclic pattern of maturity was observed for wild caught *N. cyanomos* with an abundance of ripe females from July to March and a less abundance period from April to June. But ripe females were not totally lacking during this period.

This size frequency data of ova also point to the continuous breeding of these two species. Ripening ovary of both the species contained maturing and immature eggs in large numbers. The maturing ovary of *N. cyanomos* contained only two sets of eggs and the largest group was in the maturing stage only. It may become similar to a ripening ovary as the maturation progresses and with the appearance of a new set of immature eggs at the beginning.

The size and age at first maturity for males and females of *A. sebae* and males of N. *cyanomos* are not controlled by biological factors alone. Behavioral aspects and social organisation play a major role in the sexuality of these fishes. Therefore the size at first maturity of only female *N cyanomos* was determined. Schwarz and Smith (1990) suggested that in protogynous *Dascyllus reticulatus,* all individuals above the size at first maturity are capable of changing sex, but the completion of the sex reversal is socially controlled. Thus in *N.cyanomos* also all the mature females may be capable of transforming into males.

The sexuality of both the species under study is controlled by biological as well as social aspects. The social organisation and the behavior affect the sex ratio and sexual maturity. The information on the reproductive biology from the present study can thus form the basis for the development of broodstock and breeding of the species.

5. BROOD STOCK DEVELOPMENT AND BREEDING OF SELECTED POMACENTRIDS

5.1. Introduction

Pomacentrids are generally recognised as ornamental fishes suitable for captive breeding due to their readiness to spawn in captivity, egg attaching habit and parental care of eggs. Their social organisation and behavioural peculiarities, especially those associated with spawning are unique and fascinating. Hence this group has been subjected to a lot of field studies on the behaviour and breeding pattern. The most intensively investigated members of this vast group are the anemonefishes. Formation of breeding groups, individual interactions within the group and spawning patterns are vivid in anemonefishes and consequently much research attention has been focused on different species of anemonefishes. Still, there are also many reports on the ecology and behaviour of other damselfishes.

Pomacentrids in general are inhabitants of coral reef areas and shallow rocky seas and majority of them establish and defend feeding and breeding territories (Allen, 1991). The anemonefishes are symbionts on sea anemones and the nature of symbiosis is well documented in early reports (Mariscal, 1970a ; 1970b ; 1972 ; Allen, 1972; Fautin, 1986). Certain damselfishes of the genus *Dascyllus* also exhibit association with sea anemones (Stevenson, 1963). The anemonefishes established territories around the host sea anemones and laid eggs in the vicinity of the anemones (Allen, 1972 ; Moyer and Bell, 1976 ; Ross, 1978 ; Richardson *et al.*, 1997). Territorial behaviour in many other species of pomacentrids has been studied earlier (Myrberg *et al.*, 1967 ; Swerdloff, 1970 ; Clarke, 1971 ; Boer, 1980 ; Ebersole, 1980 ; 1985 ; Khoda, 1981 ; 1984 ; Shpigel, 1982 ; Jones and Norman, 1986 ; Allen, 1991 ;

anemonefish, *A. clarkii* onset of breeding by an individual is not only determined by the age but also by the ranking in the social hierarchy (Ochi, 1986a). The sex change in anemonefishes occurs only as a best of a bad situation, thus a male which lost it's mate changes sex only when it does not get another female partner (Ochi and Yanagisawa, 1987). For breeding males which lost mates, there will be a reproductive pause due to the time taken for sex change and for the unmated males the adaptability is lost by transformation to female (Ochi, 1989b). Largest non-breeders refrained from becoming females to keep their gonads ambisexual so that they could replace either of a breeding pair. Breeding spaces are available to non-breeders only after the disappearance of one or both members of the established pairs (Ochi, 1989b). Size composition of the members in a colony and mobility also affect the pattern of pair formation in anemonefishes (Hirose, 1995). Due to the difference in timing among individuals in the development of ovarian tissues of the hermaphroditic gonads, different life history pathways have been reported in anemonefish *A. clarkii* (Hattori and Yanagisawa, 1991).

Polygynous mating system with protogynous sex change is observed in *Dascyllus reticulatus* in which larger individuals in a colony are usually males (Schwarz and Smith, 1990). Godwin (1995) suggested the effect of ecological factors and phylogenic history in the mating system of humbug damselfishes. In certain damselfishes usually one female and one male take part simultaneously in spawning eventhough more functional females are present in the colony with occasional polygyny (Goulet,1995; Schwarz, 1995). Females of *Amblyglyphydodon leucogaster* were promiscuously mating with different males at different spawnings (Goulet, 1995; 1997). The frequency of spawning and territorial defense is more when population density is high (Barnett and Pankhurst, 1996) and the frequency of agonistic behaviour will be more in captivity than in natural conditions (Cleveland, 1999).

The breeding behaviour and patterns of spawning in wild conditions have been investigated for a number species of pomacentrids, such as *Chromis multilineata* (Myrberg *et al.*, 1967), *C. caeruleus* (Swerdloff, 1970), *C. cyanea* (Boer, 1980), *C. notatus* (Ochi, 1985a; 1986b), *C. dispilus* (Tzioumis and Kingsford, 1995), *Hypsipops rubicundus* (Clarke, 1971 ; Sikkel, 1988 ; 1989 ; 1994a ; 1994b; 1995a ; 1995b ; Knapp *et al.*, 1995), *Amphiprion clarkii* (Moyer and Bell, 1976 ; Ochi, 1985b ; 1989a; 1989b), *A. melanopus* (Ross, 1978), *A. latezonatus* (Richardson *et. al.*, 1997), *A. akindynos* (Richardson *et al.*, 1997), *Acanthochromis polyacanthus* (Thresher, 1983 ; Thresher and Moyer, 1983 ; Kavanagh, 2000), *Abudefduf saxatilis* (Mochek, 1978 ; Prappas *et al.*, 1991 ; Foster, 1987), *A. troscheli* (Foster, 1987), *A. abdominalis* (Tyler and Stanton, 1995), *Stegastes altus* (Khoda, 1988), *S. partitus* (Knapp, 1993 ; Knapp *et al.*, 1995), *S. lucostictus* (Knapp *et al.*, 1995), *Amblyglyphydodon leucogaster* (Goulet, 1994 ; 1995 ; 1997 ; 1998), *Dascyllus albisella* (Danilowicz, 1995a ; 1995b), *D. marginatus* (Fricke, 1980), *Parma microlepis* (Tzioumis and Kingsford, 1995), *Microspathodon chrysurus* (Pressley, 1980), *Plectroglyphydodon johnstonianus* (Mc Donald, 1976). The effect of hormones in the reproductive behaviour and breeding was described by Pankhurst (1990; 1995), Pankhurst and Carragher (1995) for *Chromis dispilus,* Pankhurst *et al.* (1999) for *Acanthochromis polyacanthus* and Sikkel (1993) for *Hypsipops rubicundus.*

Lunar periodicity of spawning has been reported in many pomacentrid fishes such as *Amphiprion melanopus* (Ross, 1978), *Abudefduf troscheli* (Foster, 1987), *Amphiprion latezonatus* and *A. akindynos* (Richardson *et al.*, 1997). However, no lunar cyclic spawning was noted in species such as *Abudefduf saxatilis* (Mochek, 1978; Foster, 1987), *Chromis notata* (Ochi, 1986b) and *Microspathodon chrysurus* (Pressley, 1980). Females of many damsel fishes usually selected nests with younger stage eggs for spawning (Sikkel, 1988 ; 1989 ; 1994a, b ; Knapp *et al.*, 1995 ; Goulet, 1994 ; 1997). Nest quality is also a factor determining the spawning site choice by females (Sikkel, 1995b). Promiscuous mating system was observed for *Amblyglyphydodon leucogaster* females and are capable of laying new batch of eggs every second day (Goulet, 1994 ; 1997).

Knapp and Kovach (1991) and Knapp and Warner (1991) reported that courtship rate influenced spawning site choice by female *Stegastes partitus.* Females of the same species avoided nests where previous brood was lost to predators (Knapp, 1993).

Pomacentrids exhibit parental care of eggs and in most species it is reported to have done exclusively by the males till the hatching of the eggs. Biparantal care is reported from *Acanthochromis polyacanthus* (Kavanagh, 2000). Filial cannibalism by custodial males is reported from certain species (Hoelzer, 1988 ; 1995 ; Sikkel, 1994a). Eggs of all pomacentrids hatch in the night, mostly few hours after sunset except in *A. polyacanthus* where mid day hatching is reported (Kavanagh, 2000). Hatching of eggs of *A. saxatilis* was delayed by exposure to continuous light at the time of hatching (McAlary and McFarland, 1993). Predation of newly hatched larvae by the parents has been reported in *Acanthochromis polyacanthus* (Thresher, 1985).

The phenomenon of sex change exhibited by many members of the family is of special interest to researchers in marine fish breeding. The social and physiological aspects of sex change are known only for a few species of pomacentrids. Information on mating systems and breeding patterns are also scanty for many tropical damselfishes. Knowledge on these aspects is a pre requisite for their captive broodstock development. Eventhough much information in this regard is available from the wild populations in many reef areas, experimental studies under captive conditions are very few. Such studies would yield valuable information on broodstock development and breeding for the mariculture of these fishes. Therefore, the present study was undertaken in which detailed observations on the breeding group formation of two species and breeding patterns of seven species of pomacentrids were done under captivity.

5.2. Materials and Methods

The work was carried out in two steps. In the first part various aspects oh the development of broodstock from the juveniles were studied for one species of anemonefish and one species of damselfish. In the second step, observations on the breeding characteristics under captive conditions were studied for one species of anemonefish and six species of damselfishes.

5.2.1. Broodstock development

Experiments were conducted to study the pattern of breeding pair / group formation, minimum size of breeding pair / group and patterns of sex change. For anemonefishes all sub adults used in different experiments were individuals which were less than one year old and were not part of any established breeding pair and were selected from the subordinate group.

5.2.1.1. Formation of breeding group from juveniles

Patterns of growth and maturity of juvenile groups in the first year were studied for one species of anemonefish *A. sebae* which is monogamous and one species of damselfish *Neopomacentrus cyanomos* which is polygynous.

Anemonefish was reared in the Marine Ornamental Fish Hatchery, at the Vizhinjam Research Centre of CMFRI, and juveniles of almost uniform size and known age (date of hatching) were selected. Three month old fishes were collected and their total lengths were measured to the nearest millimeter after

anaesthetizing them using clove oil and alcohol (Munday and Wilson, 1997). Two glass tanks of size 90 cm × 45cm × 45 cm were used as experimental units. Both the tanks were provided with undergravel filters and host sea anemone *Stichodactyla haddoni*. Five juveniles were introduced into the first tank (Set I) and four into the second tank (Set II). The total length of fishes in both sets at the beginning of the experiment is given in Table 1. The fishes were fed with boiled mussel meat twice daily. The experiment was continued till spawning started in Set I. Fishes of set I were measured to the nearest mm after anaesthetizing, and fishes in Set II were sacrificed to examine gonads.

For the regal demoiselle, *Neopomacentrus cyanomos,* juveniles were collected from the Vizhinjam Bay. Fishes of almost equal size were selected and the total lengths were measured to the nearest mm after anesthetization. Three circular plastic containers of 60 cm diameter and 30 cm height were used as experimental units. Each unit was provided with undergravel filter and a small earthen pot as shelter. Five fishes each were introduced into each tank (Set I, Set II and Set III). The total lengths of fishes in all the sets at the beginning of the experiments are given in Table 1. They were fed with boiled mussel meat twice daily. Regular observations were made on the behaviour and social interactions in each group. The experiment was continued till spawning started in set I. The fishes in all sets were sacrificed and total length and gonadal condition were noted.

5.2.1.2. Minimum size of breeding group

Experiments were conducted to test whether two juveniles were sufficient to form a breeding group. The experiment was done for *A. sebae* and *N. cyanomos*.

Table1. Total lengths (mm) of fishes introduced for experiment on breeding group formation

Amphiprion sebae		*Neopomacentrus cyanomos*		
Set I	Set II	Set I	Set II	Set III
36	32	37	35	32
34	30	35	34	31
33	30	35	33	30
32	28	32	31	28
30	24	29	29	26
24				

Experiments on the anemonefish *A. sebae* was conducted in three rectangular FRP tanks of one tonne capacity. The tanks were provided with undergravel filters and host sea anemone *S. haddoni*. The total length and sex of fishes in each set are given in Table 2. The experiment was continued till commencement of spawning. Fishes in Set III were sacrificed to determine the gonadal condition.

Circular plastic containers of 60 cm diameter and 30 cm height were used for experiments on the regal demoiselle *N. cyanomos*. The containers were provided with undergravel filters and a small earthen pot as shelter. The length and sex of fishes in each of the three sets are given in Table 2. The fishes were sacrificed for examination of gonads after 8 months.

5.2.1.3. Sex reversal

The possibility of sex change in captive conditions and the time required for transformation were studied for *A. sebae*. Functional females were removed from three breeding pairs in one tonne rectangular FRP tanks. The lengths of the remaining functional males and sub adults in each set and the length of sub adult fish introduced are given in Table 3. The groups were kept undisturbed and observed regularly for possible spawning.

5.2.2. Captive breeding

Captive breeding patterns were studied for the anemonefish *A. sebae* and damselfishes *Pomacentrus caeruleus*, *P. pavo*, *Neopomacentrus cyanomos*, *N. nemurus*, *N. sindensis* and *Dascyllus carneus*. Adult fishes collected from wild were used as broodstock for all the seven species attempted.

Table 2. Total length and sex of *A. sebae* and *N. cyanomos* at the beginning and end of experiment for minimum breeding group size

	Initial length (mm)	Sex	Duration of Experiment (Months)	Final length (mm)	Sex	Remarks
A. sebae						
Set I	68	SA	3	71	♀	Spawned after transformation
	60	SA		64	♂	
Set II	78	♂	2.5	80	♀	"
	66	SA		67	♂	
Set III	88	♂	3	91	♀	Sacrificed and gonads were examined
	71	SA		73	♂	
N. cyanomos						
Set I	36	Juvenile	8	66	♀	Sacrificed and gonads were examined
	34	Juvenile		60	♀	
Set II	41	Juvenile	8	58	♀	"
	38	Juvenile		46	♀	
Set III	32	Juvenile	8	56	♀	"
	31	Juvenile		48	♀	

Table 3. Total length of fishes and duration of transformation in the experiment for sex change in *A. sebae*

	Length of fishes in the initial group (mm)	Sex	Length of sub adult introduced (mm)	Time taken for initiation of Spawning (Days)
Set I	86	Functional male	76	122
	54	SA		
	48	SA		
Set II	79	Functional male	68	135
	56	SA		
Set III	82	Functional male	75	61
	51	SA		

Broodstock developed from hatchery produced fishes were also used for *A. sebae, N. cyanomos* and *P. caeruleus.* For the anemonefish, observations were made for a period of 26 months and data were collected from 15 breeding pairs. The length and number of spawnings of each pair is given in Table 4. Details on the brood stock of each species is given in Table 5. The fishes were fed *ad libitum* with boiled mussel meat. Host sea anemone *S. haddoni* was provided for all anemone fish broodstock tanks. Small earthen pots were kept for damsel fishes as shelter and substratum to lay eggs. Observations were made on the spawning behaviour, frequency, time and duration of spawning, seasonality, clutch size and incubation period. Lunar periodicity of spawning was tested for *A. sebae* and *D. carneus* which exhibited distinct spawning cycles. Number of spawnings falling in each lunar quarter was counted and analysed using χ^2 test to test the null hypothesis that the number of spawnings did not vary significantly in the four lunar quarters. The number of eggs laid was estimated by counting the number of eggs in a small portion of the egg clutch of known area and multiplying it with the total area of the egg clutch (Danilowicz, 1995b). For estimating the annual fecundity of the anemone fish, only those pairs where observations were available for at least one year were selected. In the pair 2 where observations were available for two years, data on each year was taken separately. Site preference for the deposition of eggs were studied in *P. pavo* when repeated spawning occurred before the hatching of the existing clutch. The number of times the fishes spawned contiguous to the existing clutch and also as a different clutch were noted for each day after the initial spawning. The brood stock of damsel fishes were sacrificed after six months to determine the number of males and females in the group. In other groups the sex was determined by direct observation of spawning.

Table 4. Total lengths of male and female of 15 pairs of anemonefishes and number of spawnings from each pair

Pair No.	Length of male (mm)	Length of female (mm)	No. of spawnings observed
1	100	108	15
2	102	106	35
3	92	105	48
4	97	104	15
5	84	91	11
6	72	89	19
7	84	90	5
8	90	92	5
9	75	84	4
10	84	92	21
11	70	88	1
12	82	89	14
13	75	82	16
14	95	107	13
15	77	81	14

Table 5. Details of broodstock of different species of damselfishes

Species	Source	Total length (mm)	Sex	Remarks
D. carneus				
	Wild caught	74	♂	
		69	♀	
		65	♀	Died after 6 months
		58	♀	Died after 6 weeks
P. caeruleus				
	Set I (Wild caught)	76	♂	
		74	♂	
		74	♀	
		71	♀	
		70	♀	
		68	♀	
	Set II (Reared)	81	♂	
		72	♂	
		60	♀	
		54	♀	
		52	♀	
		48	♀	Not found to take part I spawning
		40	--	

		♂	
	72		
		♀	
	66		
		♀	
	61		
		♀	
	55		
		♀	
Set III (Reared)	52	--	
	49		
		--	Not found to take part in spawning
	44		
		--	
	41		
		--	
	38		
		--	
	36	—	
	29		
N. cyanomos			
	105		
		♂	
	95		
		♂	
	92		
		♀	
	85		
		♂	
	80		
		♀	
	80		All were sacrificed after 6 months
Wild caught		♀	
	76		
		♀	
	75		
		♀	
	71		
		♀	
	70		
		♀	
	70		
	67	♀	

N. sindensis			
		106	♂
		104	♂
		100	♂
		95	♀
		95	♀
	Wild caught	95	♀
		94	♀
		93	♀
		93	♀
		93	♀
		91	♀
		90	♀

All were sacrificed after 6 months

5.3 Results

5.3.1 Broodstock Development

5.3.1.1. Breeding group formation in anemonefish, A. sebae

The total length of fishes introduced in Set I and Set II at the beginning and the end of experiment are given in Table 1. The relative growth of all individuals during the study period in each set with respect to the mean initial length of the group is shown in Fig.1. The largest two fishes in each group grew faster than others and became dominant over the other individuals of the group. They chased away the smaller fishes from the anemone. These larger fishes eventually became the breeding pair in each group.

Spawning started in Set II in 12^{th} month after beginning the experiment. The largest fish was the female and next in the hierarchy was the male. In Set I the largest fish was a mature female and the next largest was a mature male. The largest fish in subordinate group was an immature male and the remaining fishes possessed indeterminate gonads.

5.3.1.2. Breeding group formation in Neopomacentrus cyanomos

The initial lengths of fishes introduced in the three sets are given in Table1. The largest fish in all the sets occupied the earthen pots provided in the tank and aggressively defended it. The establishment of territories occurred within two days after introducing them to the system. Smaller fishes took shelter beneath small coral pieces used for the under gravel filter but were not vigorously defending it .The largest fish dominated the groups and grew faster than others (Fig. 2).

Fig.1. Relative growth in percentage to mean initial length of the juvenile group of *A. sebae* after one year

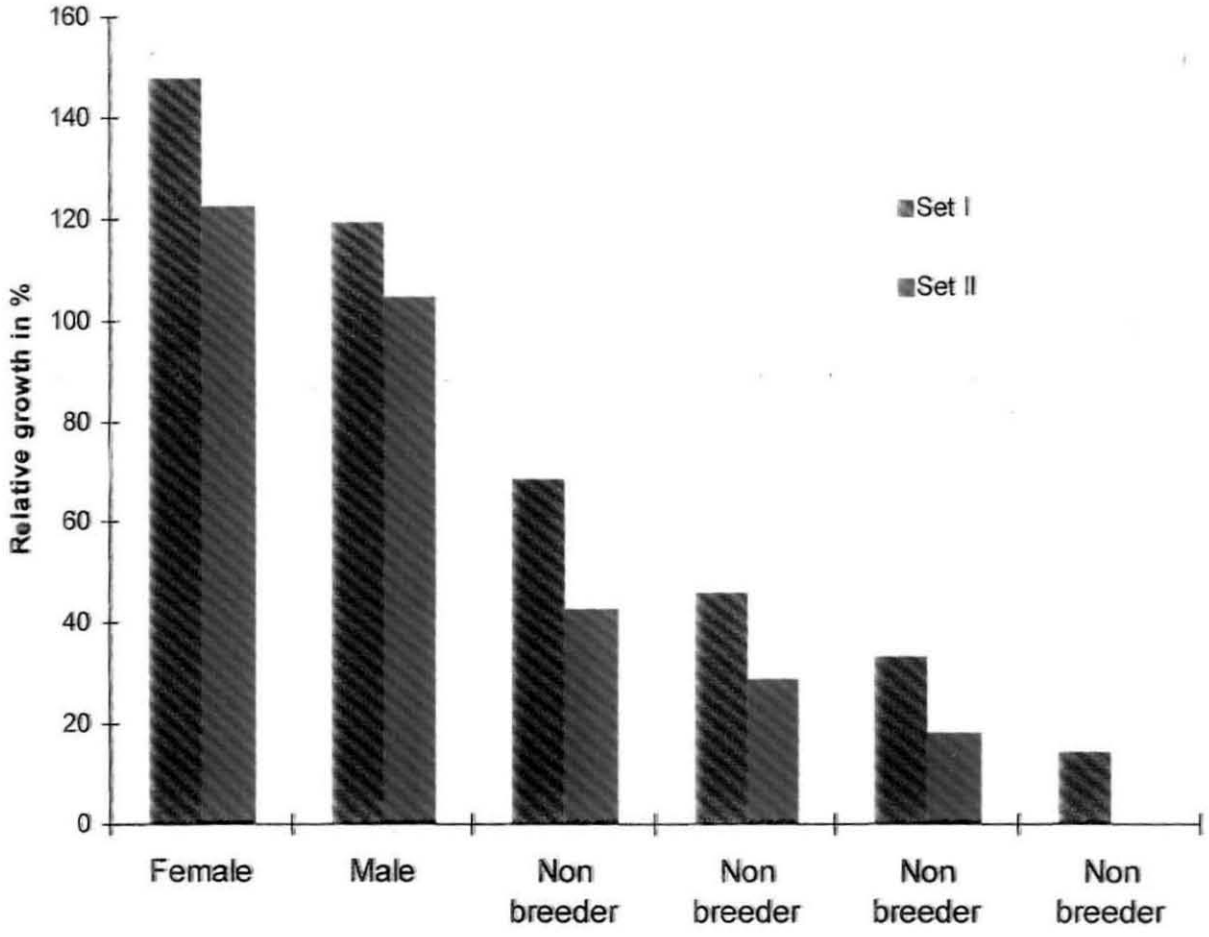
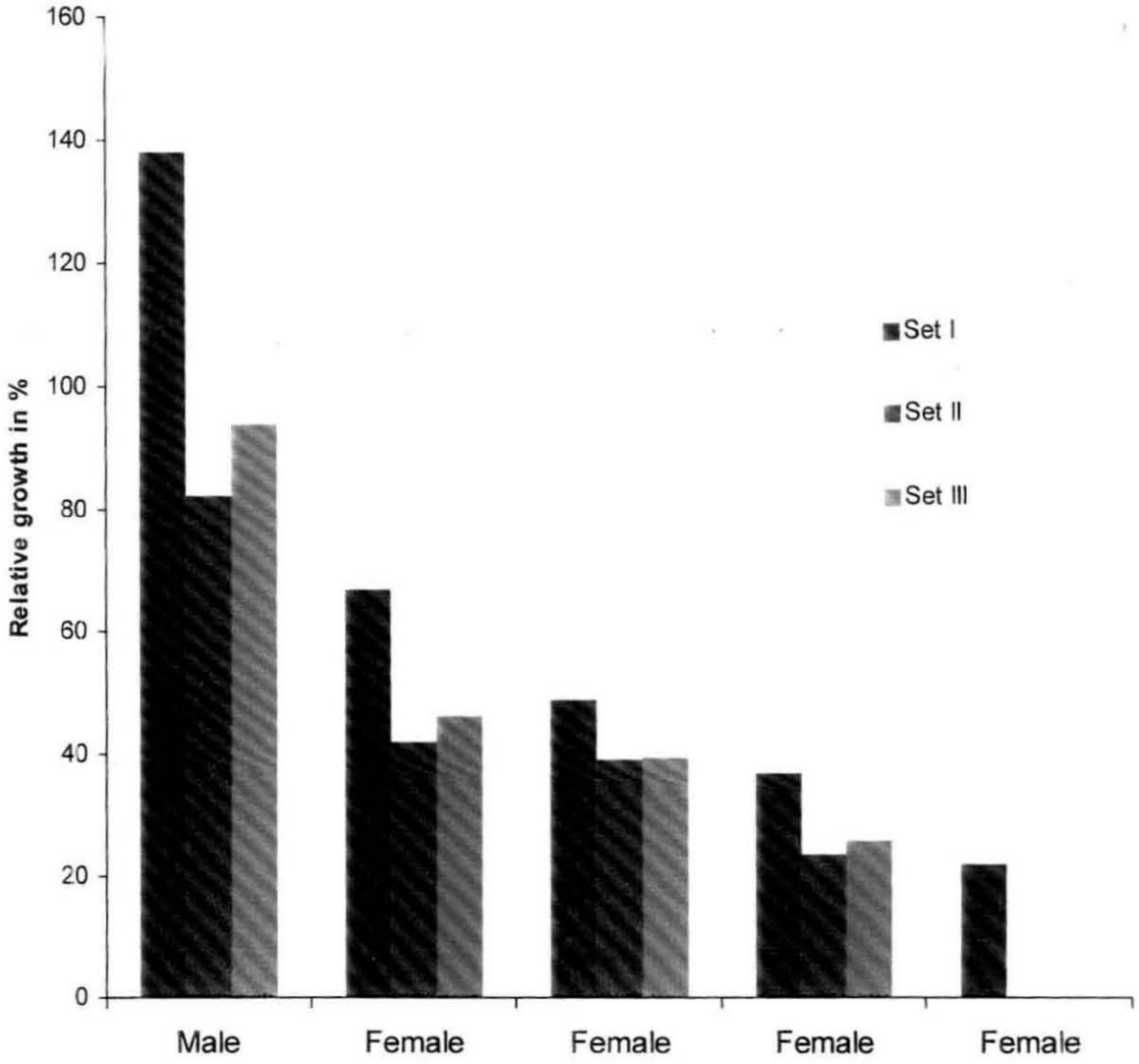


Fig. 2. Relative growth in percentage to mean initial length of the juvenile group of *N. cyanomos* after eight months



Spawning started in Set I eight months after introducing them to the system. The largest fish in this set was functional male and all other possessed ovaries with ripe ova. Similarly in Set II and Set III the largest fishes were mature males. In both sets the largest two of the remaining fishes were mature females and the smallest fishes had stage III ovaries.

5.3.1.3. Minimum size of breeding groups in anemone fish

Spawning occurred in Set I and Set II. In Set I first spawning occurred three months after introducing into the experimental unit and the fishes were 13 months old. At the time of spawning the total lengths were 71 mm and 64 mm. In Set II spawning started after two and a half months (Table 2). The larger fish was the female and the other a male. A male to female transformation had occurred to the larger fish. There was no spawning in Set II upto three months after introduction. On examination of the gonads the larger fish was found to possess ovary with ripe eggs and the smaller one was a male. Here also a male to female transformation was seen in the larger fish.

5.3.1.4. Minimum breeding group size in **Neopomacentrus cyanomos**

The total length of fishes at the end of the experiment is given in Table 2. Both fishes in Set I possessed ripe ovaries. In Set II and Set III both fishes possessed stage III ovaries. The aggression by larger fishes was less in all three sets.

5.3.1.5. Sex reversal in the anemone fish, **A. sebae**

Spawning occurred in all the three sets. But the time taken for initiation of spawning varied widely in the three sets. The details of the size and duration taken to initiate spawning in all the sets are given in Table 3. In Set I and Set II spawning commenced after four months. In set III it took only two months to start spawning. In all the sets the functional male transformed into female and laid eggs. The newly introduced sub adults which were larger than those present in the group became functional males in all the sets.

5.3.2 Captive breeding

5.3.2.1 Amphiprion sebae

Observation for 25 month period was taken on the breeding patterns. During this time 236 spawnings were noted from 15 breeding pairs. The period of observation and duration of spawning of the 15 pairs is shown in Fig. 3.

Breeding behaviour :The breeding activity commenced with cleaning of the nesting site by both male and female. Sometimes this behaviour commenced on the previous day of spawning. Just prior to spawning the fishes exhibited parallel swimming usually with belly touching, and biting on the substratum and tentacles of anemone with increased agonistic behaviour. The females started attaching eggs to the substratum, immediately followed by fertilization by the male. The spawning of the anemonefish is shown in Plate 1. The spawning occurred between 09. 00 am and 12. 00 noon and lasted for 1 to 1.5 hours. The eggs were yellow or orange in colour initially. They turned to light grey colour on third day and the silvery colour of eyespot was clearly visible from seventh day.

Parental Care: Both male and females took part in fanning and caring but the male predominated in the activity. They also removed dead eggs from the clutch.

Fig 3. Period of spawning of the 15 pairs of Anemonefishes

(Months along longitudinal axis and Pair no. along vertical axis, Continuous lines indicate Spawning phase, Dotted lines Resting Phase, and large black dots Loss of breeders due to death)

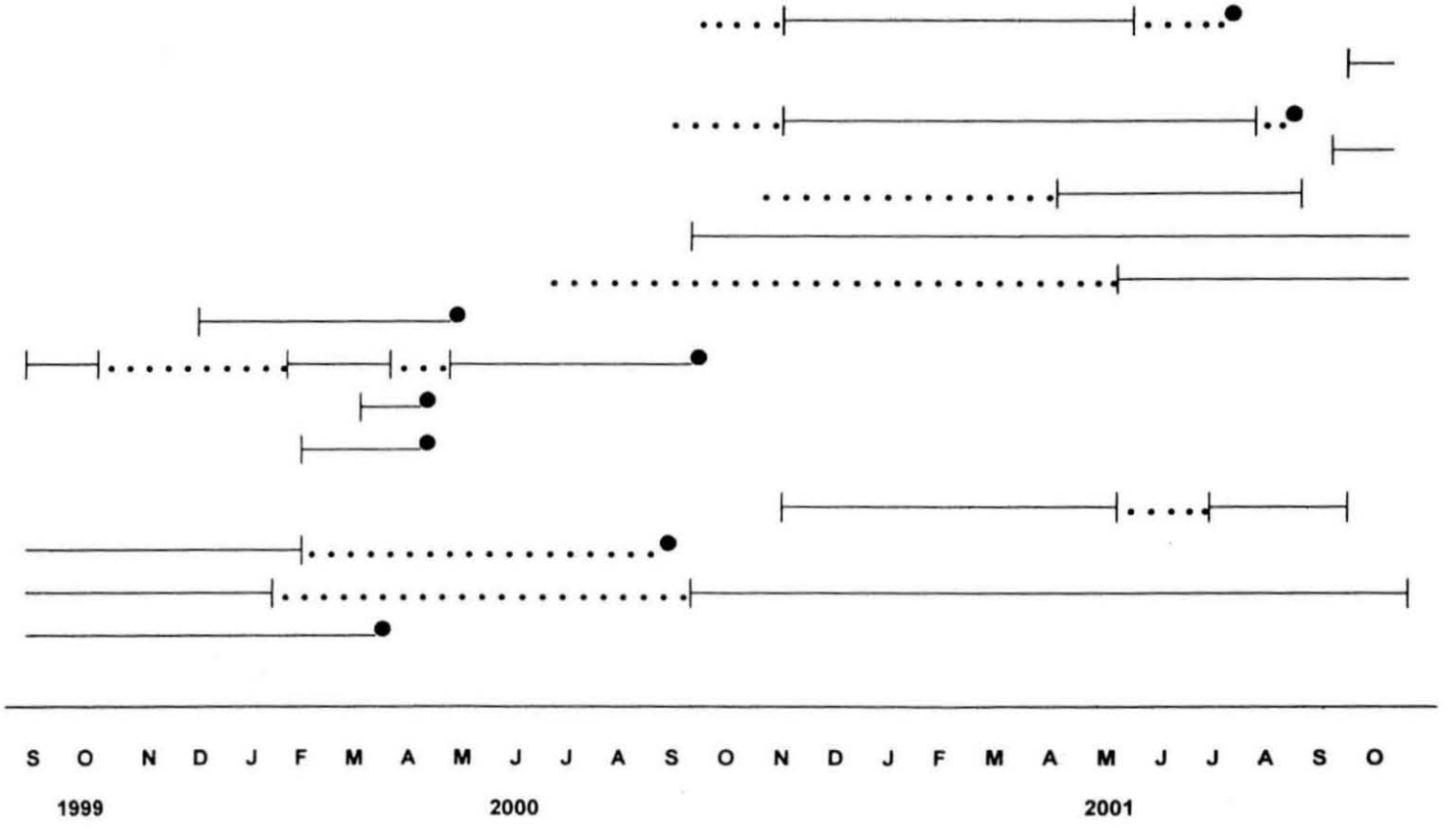
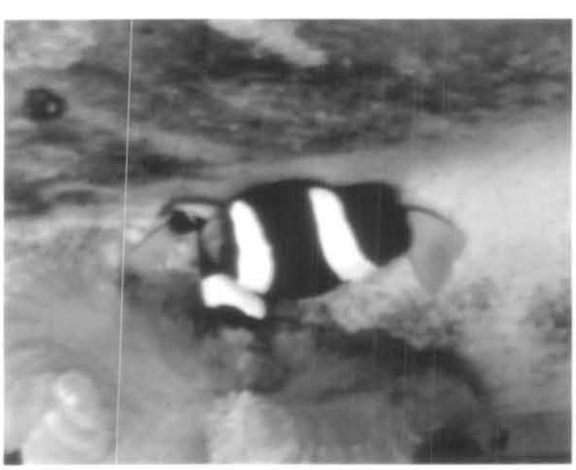
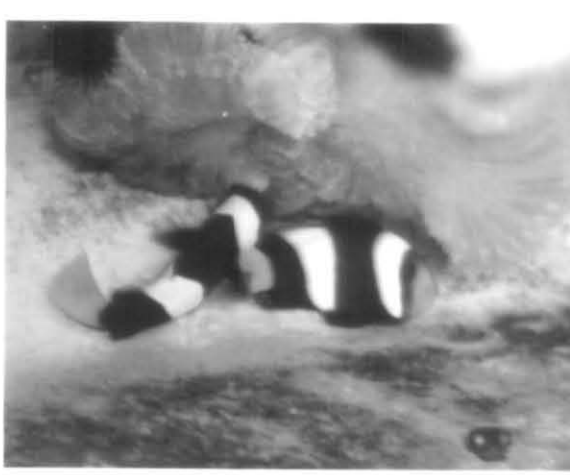


PLATE 1. SPAWNING ACTIVITY AND NEST GUARDING IN *A. SEBAE*





Incubation Period: The eggs usually hatched in the evening of the 7th and 8th day after laying. The incubation period was variable between 5.5 and 9.5 days and the monthly variation in incubation period is depicted in Fig 4. Shorter incubation period of 5.5 and 6.5 days occurred more frequently in the months of February to June and longer incubation periods are seen in the remaining months except October.

Hatching: Hatching took place in the evening about 2 to 3 hours after sunset. Partial hatching occurred on some occasions where part of the egg clutch hatched in one day and the remaining on the next day.

Spawning frequency and periodicity: The interval between two successive spawnings generally varied between 9 and12 days. The frequency distribution of duration of spawning cycles is given in Fig. 5. Long intervals of more than 20 days were excluded because they were usually due to disease and physical stress and were not part of the normal breeding pattern. Long intervals in spawning without any physical problem to the breeder were taken as the resting phase between two breeding phases. The resting phases varied between 4 and 7 months and the continuous spawning phase varied between 6 and14 months. There was no distinct seasonality in spawning eventhough there was a reduction in the number of spawnings and number of eggs laid in June and July. Number of spawnings and the number of eggs laid in each month are shown in Fig 6. The number of spawnings with respect to lunar cycle is shown in Fig. 7. The spawning did not follow any distinct lunar phases and the number of spawning was not significantly different in the four lunar quarters ($df = 3$, $\chi^2 = 5.31$, $p < 00.5$).

Fecundity: The average number of eggs laid in one spawning was 569 ± 11.81 ($n = 236$). The average annual fecundity of the selected pairs was 10231 ± 1473.02 ($n = 183$) eggs and the average number of spawning in a year was 18.3 ± 2.01.

Fig. 4. Monthly variation in incubation period in *A. sebae*

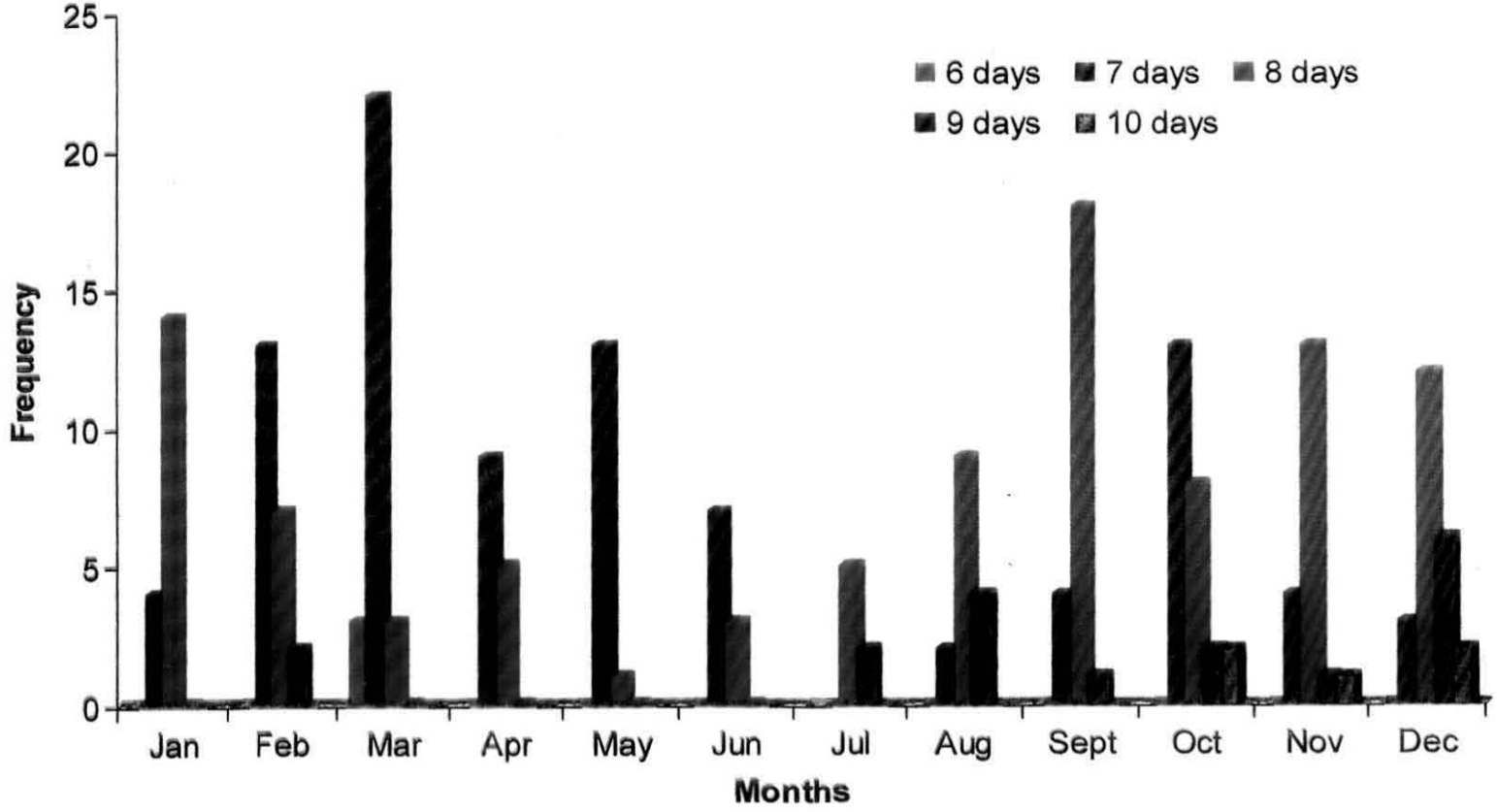


Fig. 5. Frequency distribution of spawning cycles in *A. sebae*

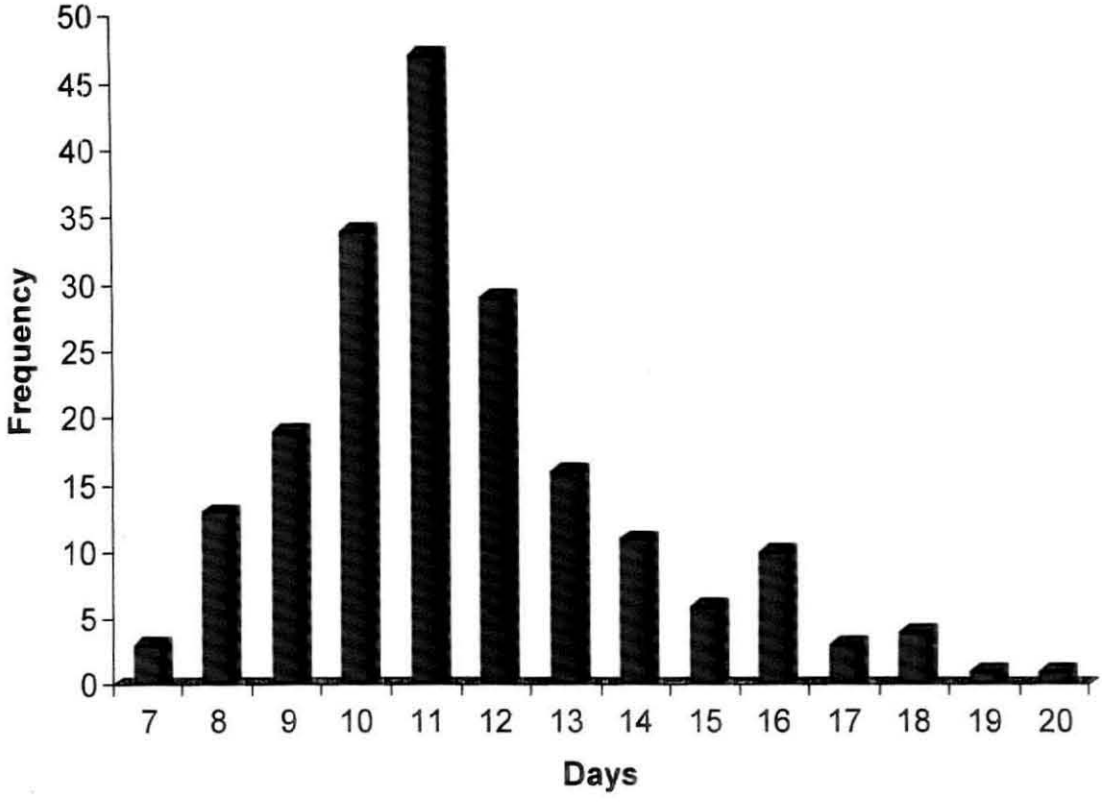


Fig. 6. Number of spawnings and number of eggs laid in each month by *A. sebae*

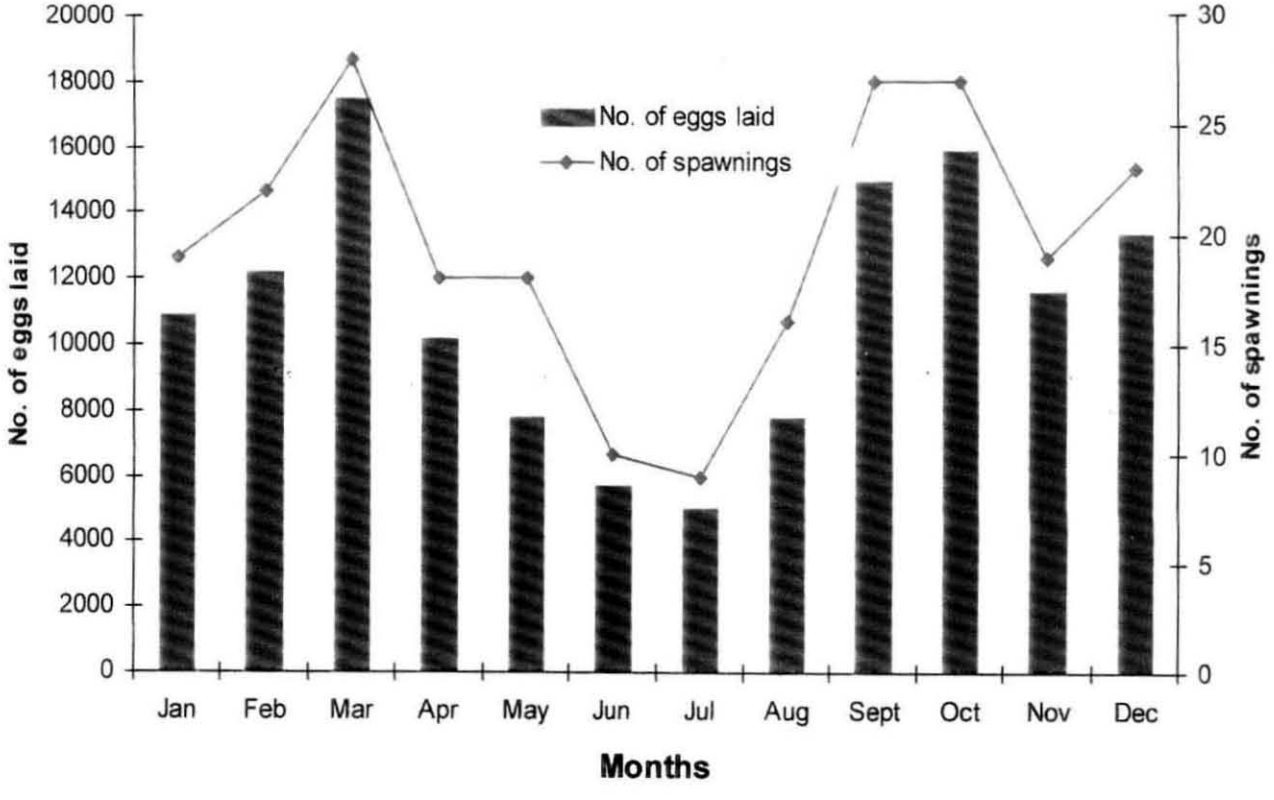
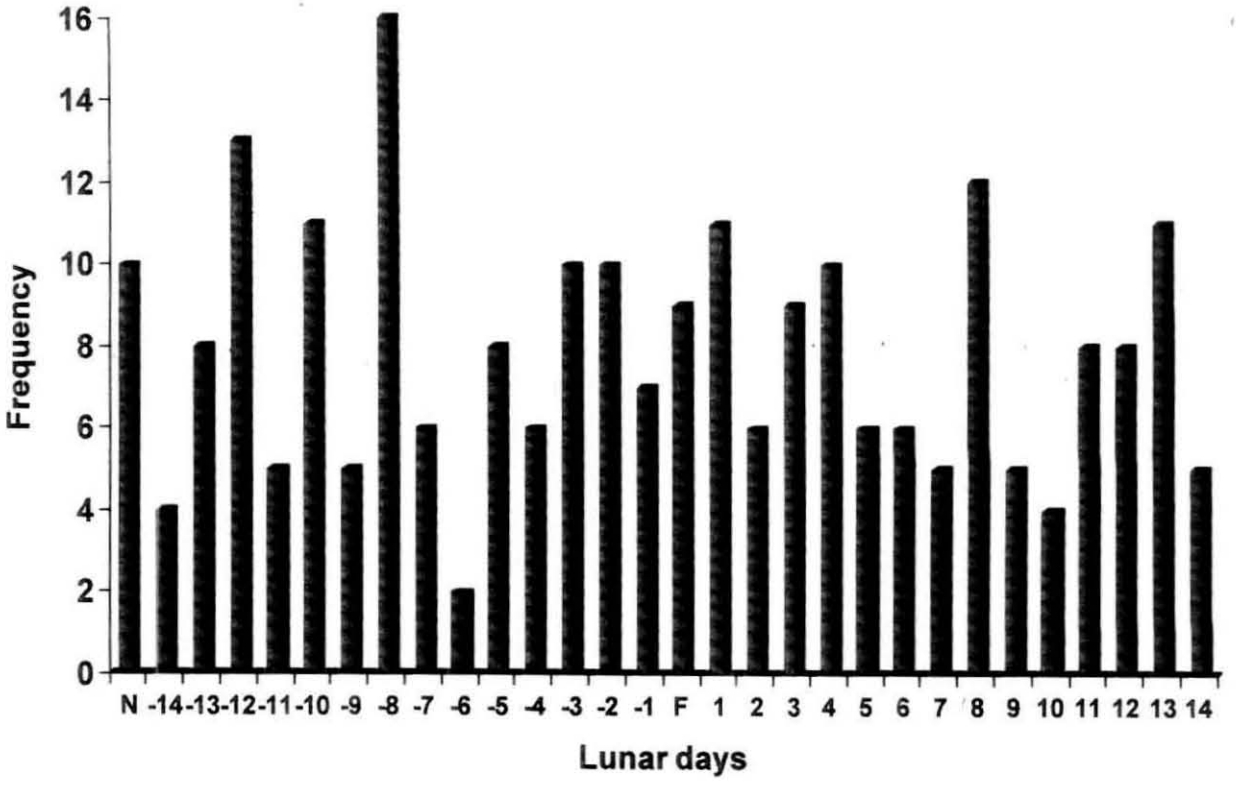


Fig. 7 . Number of spawning with respect to lunar cycle in *A. sebae*



N - New moon F - Full moon

5.3.2.2. Dascyllus carneus

During the 16 month study period the fish spawned 36 times with an average of 2.25 spawnings per month. The number of spawnings and the total number of eggs laid in each month is shown in Fig 8. The number of spawnings ranged from 0 to 4 in a month and the average number of eggs laid per spawning was 4065 ± 421.62. The spawnings did not show any lunar periodicity and the number of spawnings was not significantly different in four lunar quarters (df = 3, $\chi^2 = 1.1$, $p < 0.05$). The number of spawnings with respect to lunar cycle is given in Fig 9. The spawning cycle was mostly of 12 days and the frequency distribution of spawning intervals between two successive spawnings is shown in Fig. 10.

Breeding behaviour: There were one male and two females in the breeding group. Both fishes died before the onset of spawning were mature females. The spawnings occurred between 0900 am and 1100 am and lasted for 1.5 – 2 hours. At the time of breeding the colour of the fishes especially the male showed rapid change to dark brown and violet. Usually one female and male took part in the spawning. First the females attached the eggs to the substratum which was immediately followed by fertilization by the male. Parental care was exclusively done by the male till hatching. The eggs hatched in the evening of the third day immediately after the sunset and incubation period was two and a half days.

5.3.2.3. Pomacentrus pavo

Spawning started two months after introducing them to the breeding tank. Spawning was almost continuous throughout the study period. The largest fish was the nest guarding male and the others were females. One fish died after 7 months and was a mature female. The number of spawning and the number of eggs laid during the study period are shown in Fig. 11. Multiple spawning was noticed 77 times. A maximum of two developmental stages was noticed among

Fig 8. Number of spawnings and number of eggs laid in each month by *D.carneus*

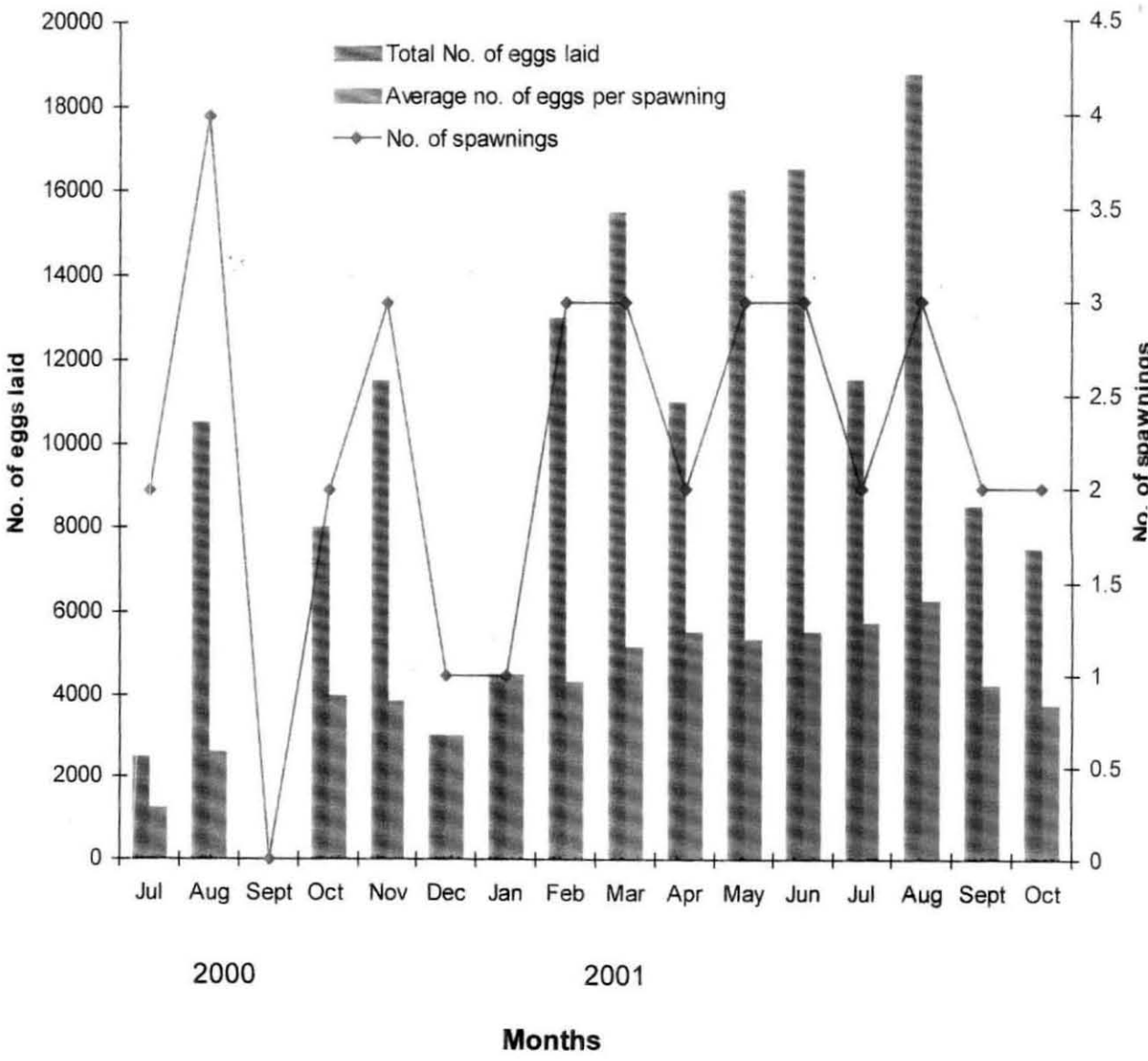
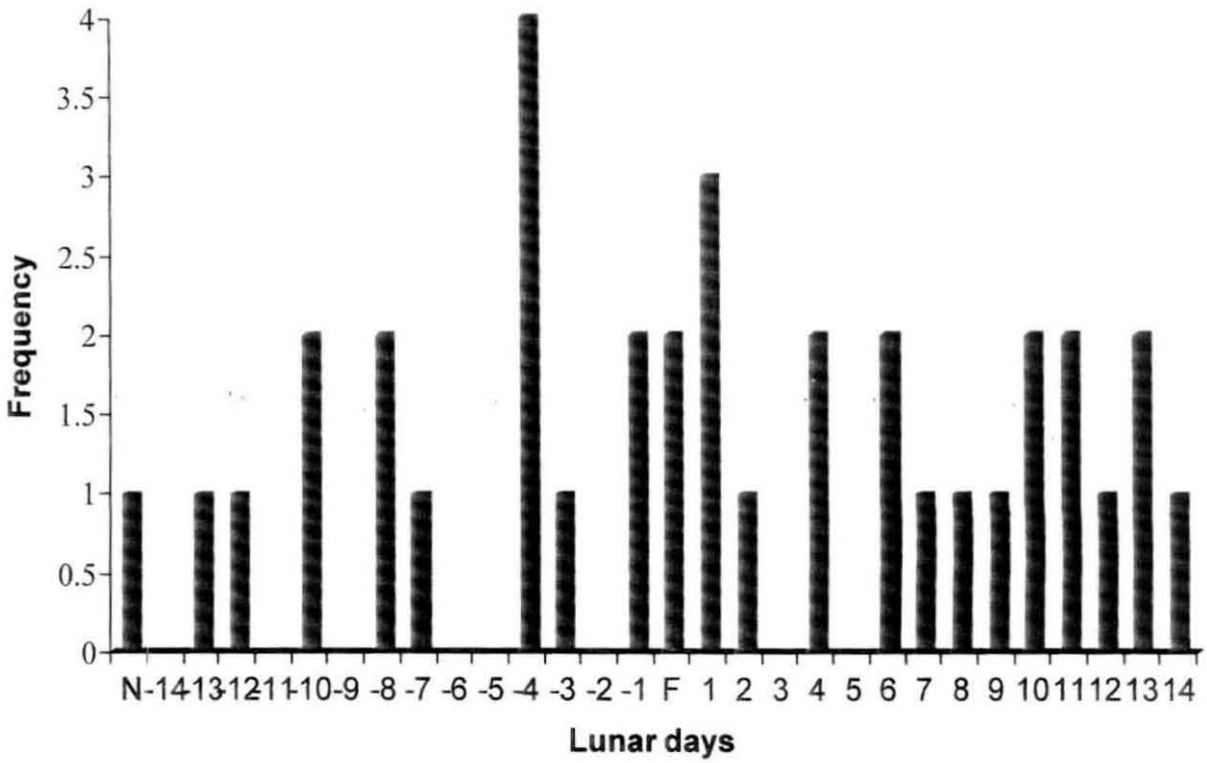


Fig 9. Number of spawnings in *D. carneus* with respect to lunar cycle



F - Full moon N - New moon

Fig 10. Frequency distribution in nesting cycles of *D. carneus*

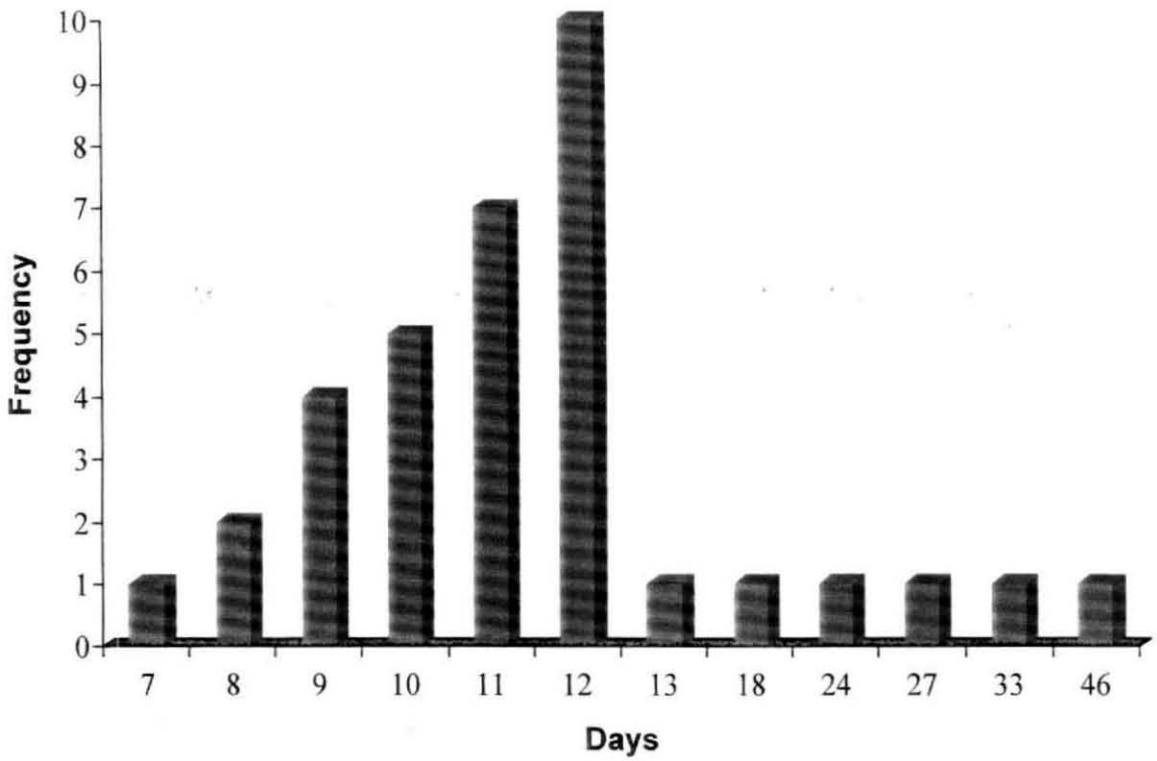
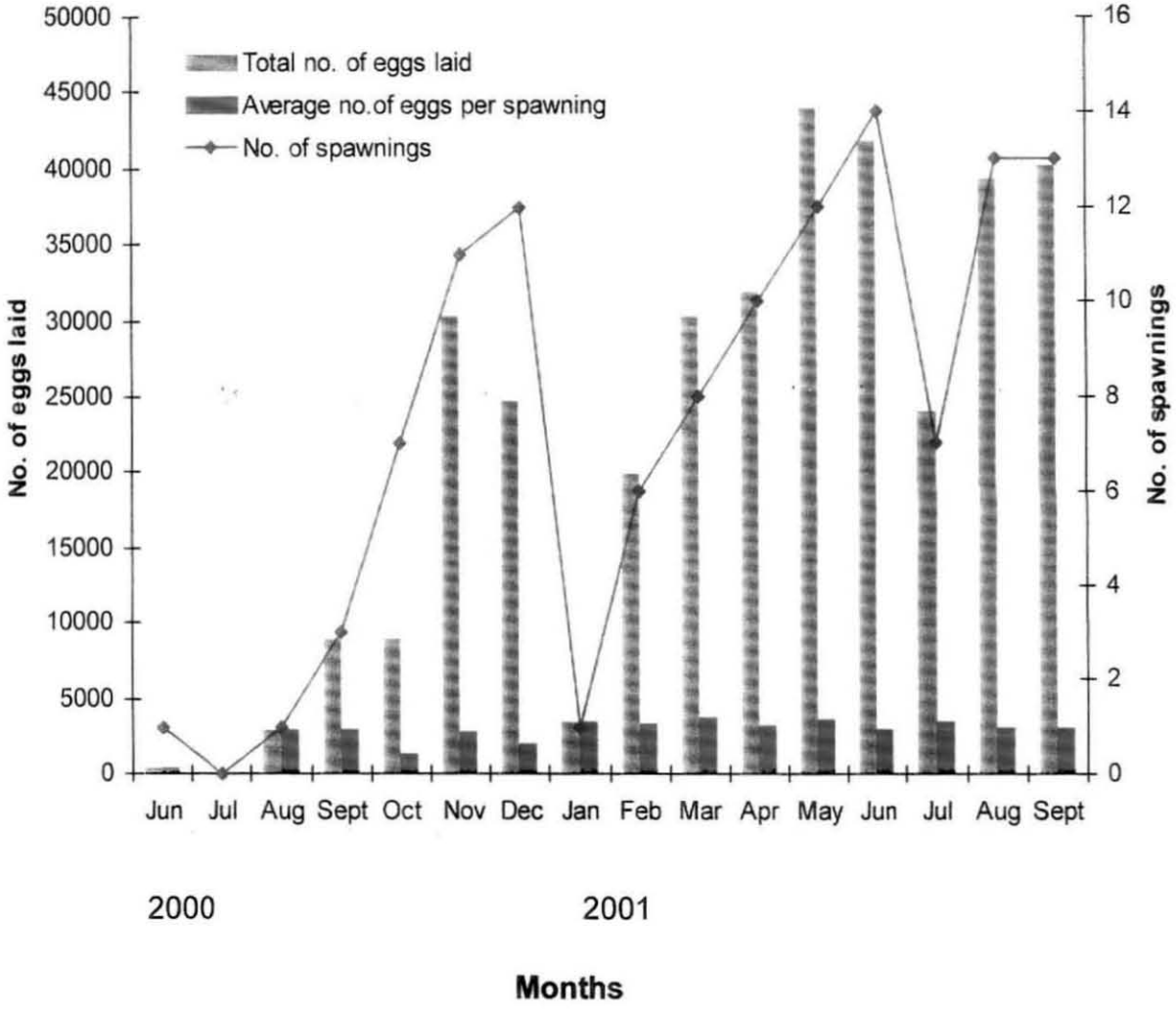


Fig 11. Number of spawnings and number of eggs laid in each month by *P. pavo*



the clutches of eggs simultaneously. The number of times the females spawned in the same clutch contiguous to the existing clutch and as a different clutch for different days is given in Table 6.

Timing and frequency : Spawning usually took place early in the morning. Occasionally spawning took place in late night hours. Spawning was rather continuous during the 16 month period. The spawning frequency and the number of eggs laid were very low initially but gradually increased. The number of spawnings ranged from 0 to 14 with an average of 7.4 ± 1.24 spawning per month. Usually the spawning occurred is every 3 - 4 days during normal breeding periods. The colour of male during courtship and brooding changed between dark blue and violet.

Clutch size :The number of eggs laid in a spawning was 400 in the initially. Afterwards it increased to a maximum of 3788, with an average of 2656 ± 78.74 eggs per spawning. Clutch size was fairly consistent after the initial spawning. During the 16-month period the total number of eggs laid was estimated to be 327450.

Incubation period and hatching :The incubation period was four days generally but occasionally extended to the fifth day. Hatching took place in the evening 1 to2 hours after sun set.

5.3.2.4. Pomacentrus caeruleus

Spawning commenced in Set II when the fishes were 9 months old, and in Set III when they were12 months old. The total lengths of fishes at the time of spawning are given in Table 5. In Set I the largest two fishes guarded the territories containing eggs and both were functional males. The smaller three fishes were functional females and took part in spawning. In Set II the egg were found with the largest two fishes both of which were functional males. The nest of these fishes was about 1 m apart. The number of functional females could not be

Table 6. Site preference by *P. pavo* for deposition of second egg clutch before the hatching of the previous clutch

Days after first spawning	Contiguous to the existing clutch	Different clutch
1	32	4
2	2	10
3	0	29

clearly ascertained. In Set I simultaneous occurrence of more than two different stage eggs were rare, but it was common in Set III where 2 to3 different staged eggs were frequently observed in the same nest. The number of eggs laid in a single spawning was 2867 ±137.21.

Timing and frequency :The egg laying was observed in the morning hours, usually between 08. 00 am and 11. 00 am. Polygynous mating was observed on many occasions. Egg laying was noted on every day or every alternate days in Set III after the initiation of spawning. In Set I the spawning occurred occasionally in 3 - 4 days intervals on the nest of a male.

Incubation period and hatching: Incubation period was mostly of four days, rarely extended to the fifth day. Hatching took place 1 to 2 hours after sunset.

5.3.2.5. Neopomacentrus cyanomos

Fishes in Set I spawned for the fist time when they were 9 months old. Eggs were found in the territory of the largest fish and that was a functional male. In Set I there were three males and the remaining were females. Spawning started within the first week after introducing them into the broodstock tank. Multiple clutches were guarded by the males many times during the study period. Egg laying was continuous throughout. The males guarded eggs of two or more different age groups simultaneously. Average number of eggs in a single spawning was 3611 ± 203.11.

Incubation period and hatching : Eggs hatched in the 4^{th} day evening about 1 – 2 hrs after the sunset. Hatching rarely extended to the fifth day.

5.3.2.6. Neopomacentrus nemurus

Continuous spawning was observed in *N. nemurus* for the three months. Examination of gonads showed that the largest three fishes were functional males and the remaining were females. Usually each male guarded nests with two or more different developmental stage eggs simultaneously. Average number of eggs in a single spawning was 2788 ± 282.24. Egg laying was observed between 08. 00 am and 10. 00 am. Hatching took place in the fourth day evening 1 to 2 hours after sunset

5.3.2.7. Neopomacentrus sindensis

The breeding groups included three males and seven females. The breeding was continuous for two months and afterwards egg laying was not noticed for four months. On examination of the gonads, it was found that all the females were mature. The nest of males contained eggs of 2 or 3 different developmental stages. The average number of eggs laid in a spawning was 4912 ± 276.74. Egg laying was during early morning and hatching on the fourth day evening 1 to 2 hours after sunset

5.4. Discussion

The pattern of brood stock development and breeding by different species in the present study was consistent with the general pattern of pomacentrids. Soon after the settlement pomacentrid juveniles establish social hierarchy and the post settlement growth of the juveniles is affected by the aggressive interaction among the conspecifics of the same colony (Ochi, 1986a ;

Booth, 1995). In the anemonefishes the initiation of breeding by individual fish is not only determined by size but also by the ranking in the social hierarchy (Ochi, 1986a). In the polygynous *Dascyllus albisella* the time taken to reach maturity decreases for juveniles settled on coral heads without conspecific adults (Booth, 1995).

Amphiprion sebae and *Neopomacentrus cyanomos* in the present study exhibited differential growth pattern as a result of aggressive inhibition. In anemonefish, the two breeders grew to a significantly larger size than the remaining fishes. Also only two fishes from each set were sexually mature. This was evident from the gonad examination in Set II and direct observation of breeding in Set I. Colonies of *A. clarkii* were reported to consist of a pair of sexually mature fishes and a group of sub adults (Moyer and Bell, 1976 ; Ochi, 1989a). The differential growth due to the aggressive inhibition of the larger fishes were prominent in captivity as there was little chance for the smaller fishes to escape from the inhibition and get access to more food. Similar observations of social behaviour had been reported for other anemone fishes which exhibited distinct social hierarchies by aggressive dominance of the larger fishes and resultant monogamous mating system (Fricke and Fricke, 1977). Ross (1990) suggested that monogamy is more adaptable for fishes with non permeable social groups where possibility for movement between social groups was low due to their scattered and unpredicted distribution. But occasional polygamy is observed in anemonefish *A. clarkii* in areas of rich abundance of host anemones and pairs establish contiguous territories which allow movement (Ochi, 1989a) and movement in turn affects the pair formation in anemone fishes (Hirose, 1995)

Polygyny is the predicted mating system in permeable social groups where movements between groups is possible (Ross; 1990). *Neopomacentrus cyanomos* in the present study exhibited polygynous mating system. In all the three sets only one male was formed and all the other fishes were mature or maturing females. The differential growth between males and subordinate

females was not as prominent as observed in anemone fishes. In all the groups, the largest fish was a male and it dominated the group eventhough the subordinate fishes established separate shelters. The juvenile which occupied the earthen pot initially became the male in all sets. Thus the social interactions leading to the formation of breeding group started immediately after settlement. Fricke (1980) reported that the group size and number of sexually active males in a group are determined by the number of available hiding places on the coral. In the present study group size and number of hiding places were constant. Therefore in the limited space of the container it might have been possible for the larger fish to control mating system and prevent the formation of further functional males.

The minimum number of individuals required to form a breeding group vary among species (Shapiro, 1984). It is also an important aspect from the mariculture point of view. For the monogamous anemonefish *A. sebae* in the present study, two fishes were sufficient form a breeding pair and this is the same for other anemonefishes as well (Shapiro, 1984). For the demoiselle, *N. cyanomos* it was evident that two juveniles did not form a breeding pair, instead both of them were females in all trials. For *N. cyanomos* a male was present in Set II and Set III of the breeding group formation experiments where only four fishes were present from three weeks after the beginning of the experiment. But it is not clear whether the minimum number is three. Also it is not clear from this study whether these two species are monandric or diandric. But it is evident that polygyny is favoured in *N. cyanomos* and monogamy in *A. sebae*.

Phenomenon of sex reversal is observed in many species of anemonefishes (Fricke and Fricke, 1977; Moyer and Nakazono, 1978; Ochi and Yanagisawa, 1987; Ochi, 1989a). Sex reversal to males was observed on five occasions in the present study : three in the experiments set for the sex reversal and two in the Set II and Set III of the experiments for determining the minimum size of breeding group. Ochi (1989a) and Yanagisawa and Ochi (1986) observed that only those

males which could not find a functional female partner changed sex in natural conditions. The possible reason is that once a male is transformed into a female, the adaptability is lost because reverse transformation is not present in anemone fishes (Fricke and Fricke, 1977; Kuwamura and Nakashima, 1998). In all the present experimental units subadults smaller than the functional males were introduced and that might have necessitated a sex change in males in all trials. The time gap between the introduction of sub adults and first spawning after transformation ranged between 2 and 5 months and it is consistent with the reports of Ochi (1989a) where it ranged from 63 to 355 days. Godwin and Thomas (1993) reported a significant increase of female hormone estradiol 17β from normal male levels to males whose female partners were removed, from 20 days after removal. The transformation must been quick to avoid a reproductive windfall and to prevent intrusion by other functional conspecifics (Ross, 1990).

The breeding behavior of all the species observed did not vary much from those described in earlier accounts. The reproductive behaviour of pomacentrids has five major steps - establishment of territory, selection of nest site within the territory, preparation of nest site, courtship and pair formation, spawning, fertilisation and parental care (Allen, 1972). Descriptions of breeding behavior of anemone fishes (Allen, 1972 ; 1991 ; Ross, 1978 ; Ochi, 1989a ; 1989b; 1985b; Richardson *et al.,* 1997 ; Moyer and Bell, 1976), *Dascyllus reticulatus* (Schwarz and Smith, 1990), *D. albisella* (Danilowicz, 1995b), *Chromis caeruleus* (Swerdloff, 1970) *C. multilineata* (Myrberg *et al.*, 1967) also confirms this general behaviour. Some species such as *Chromis multilineata* (Myrberg *et al.*, 1967) were territorial only at the time of spawning. Moyer and Bell (1976) opined that for *A. clarkii* the courtship behaviour included nest cleaning, sometimes with bodies touching and nipping at the tentacles of sea anemones. They regarded other behavioural aspects such as head shaking, dorsal and ventral leanings and jaw clicking as agonistic behaviour. Knapp and Warner (1991) and Knapp and Kovach (1991) regarded the vertical 'dives' by males as the usual courtship behaviour in *Stegastes partitus*. Such vertical dives were performed by the males of all the species studied. For *D. carneus, N. cyanomos,*

N. nemurus and *P. caeruleus* polygynous mating was noted on many occasions. In the wild population of *Amblyglyphydodon leucogaster* (Goulet, 1995) and *Dascyllus reticulatus* (Schwarz, 1995) males also exhibited similar patterns by spawning with a single female usually, with occasional polygyny. All the fishes studied lacked distinct sexual dichromatism. The colour changes observed during spawning in males especially of *D. carneus* and *P. pavo* were quick and temporary.

Two damselfish species *viz. N. cyanomos* and *P. caeruleus* in the present study spawned in the first year. *N. cyanomos* spawned for the first time when they were 9 months old. First breeding occurred in *P. caeruleus* when they were 9 months old in Set I and 12 months old in Set II. *A. sebae* bred for the first time when they were 13 months old in Set I of the experiment to determine the minimum size of breeding group and at the age of 15 months in Set I of the experiment to study the breeding group formation. But in the case of temperate populations of *A. clarkii* maturity is achieved when they were 3 years old (Ochi, 1986b). Information on other tropical and temperate pomacentrids is scanty in this aspect.

None of the three species on which round the year observations were made has shown any marked seasonality in spawning. But there was a decline in the number of spawnings and number of eggs laid during monsoon months for *A. sebae*. In case of *D. carneus* there was a short gap in spawning in December 2000 to January 2001 and thereafter consistently laid eggs despite the loss of a functional female in September 2001. The pause in December – January can be regarded as a short resting period between two long and intense spawning periods. Also there was a stoppage of spawning in September 2000 due to the physical stress of transfer from one tank to another and the time taken for acclimatization. Danilowicz (1995a) reported that the spawning in *D. albisella* was affected by temperature. But for *D. carneus* it was seen that the spawning was rather continuous round the year. A similar pattern was exhibited by *P. pavo*.

The number of spawnings per month was low initially and increased afterwards. A reproductive pause was seen for this species also during January 2001.

There are many reports of preferences of the female damsel fishes towards males with nests containing eggs of early stages (Sikkel, 1988 ; 1989 ; Knapp *et al.*, 1995 ; Goulet, 1994 ; 1995 ; 1998). A similar preference was observed in *P. pavo* female. When second egg laying was noted on the next day of the first spawning it was mostly contiguous to the existing clutch, whereas when spawned on third or fourth day after the first spawning, it was mostly laid as a separate clutch. There was no choice of males to females as a single male was present in the system and thus a within nest preference was observed where the females avoided laying eggs contiguous to third or fourth day eggs. In the wild populations of *Stegastes partituts,* the females selected males depending on the courtship rates (Knapp and Warner, 1991 ; Knapp and Kovach, 1991) and there was no choice depending on the male size or brood size of males (Knapp and Warner, 1991).

The time of egg laying and hatching did not show any marked deviation from the general nature. The incubation period showed marginal variation during the study period for all species except *D. carneus* where it was 2.5 days. For all the other damsel fishes it was mostly 3.5 days but occasionally extended for one more day. In *A. sebae* the incubation period varied widely from 5.5 to 9.5 days but was mostly of 6.5 or 7.5 days. The anemone fish eggs usually hatch on the seventh day (Allen, 1972). The incubation period of *A. latezonatus* and *A. akindynos* ranged from 8 to 10 days (Richardson *et al.*, 1997) and eggs of *A. melanopus* hatched on the seventh or eighth day (Ross, 1978). From Fig. 2 it can be seen that the incubation period was generally shorter (5.5 or 6.5 days) during the months of February to June and longer during July to January. Lower water temperature may be one of the reasons for this delayed hatching as the longer incubation periods coincided with months in which water temperature was generally low. In *D. albisella* the incubation period was 3.5 days (Danilowicz and

Brown, 1992 ; Danilowicz, 1995b), in *D. aruanus* it was 3.5 days (Danilowicz and Brown,1992), in *Stegastes partitus* it was 3.5 days (Robertson *et al.*, 1988) and in *Amblyglyphydodon leucogaster* it was 5 to 8 days (Goulet, 1995). Incubation period as high as 12 to 23 days was noted in *Hypsypops rubicundus* (Sikkel, 1988 ; 1989).

None of the above species showed any significant lunar periodicity of spawning. It was evident from the results of the χ^2 test for *A. sebae* and *D. carneus*. In the case of *P. pavo*, generally continuous spawning occurred at 2 to 5 days interval. Lunar cyclic spawning was observed for many species such as *Amphiprion melanopus* (Ross, 1978), *A. latezonatus* and *A. akindynos* (Richardson *et al.*, 1997), *Abudefduf troscheli* (Foster, 1987) and *Stegastes partitus* (Robertson *et al.*, 1988). Lunar cyclic spawning pattern was noted in *Abudefduf abdominalis* during periods of reduced food abundance (Tyler and Stanton, 1995). But for many other species there was no lunar component in spawning. They include *Abudefduf saxatilis* (Foster, 1987 ; Mochek, 1978), *Chromis notata* (Ochi, 1986b), *Microspathodon chrysurus* (Pressley, 1980), *Amblyglyphydodon leucogaster* (Goulet, 1995) and *Dascyllus albisella* (Danilowicz, 1995b).

Spawning interval was noted for the two species, *A. sebae* and *D. carneus* which exhibited distinct spawning cycles. For *A. sebae* the spawning cycle was mostly of 9 to 12 days throughout the active spawning period. In the case of *D. carneus* the spawning cycle was mostly of 12 days. A six day spawning cycle was observed in the wild populations of *D. albisella* (Danilowicz, 1995b).

Annual fecundity of *A. sebae* varied from 4575 to 21125 eggs. Wide variation in annual fecundity was observed for *A. latezonatus* and *A. akindynos* (Richardson *et al.*, 1997). The average clutch size of various species of anemone

fishes ranged from 400 to 2500 (Richardson *et al.*, 1997) and for *A. sebae* in the present study it ranged from 300 to 1450. The clutch size was lower for initial few spawnings and later stabilized at 400 to 750 eggs.

In *D. carneus* the average clutch size was much lower than 22000 reported for wild population of *D. albisella* (Danilowicz, 1995b). In the wild population there are chances of eggs from many females in a egg clutch and it is not ruled out for *D. albisella*. In the present study there were only two females present for most of the period of observation and only one in the last two months. This could be one of the reasons for the reduced clutch size. The average clutch size of all other species was almost similar and comparable to the fecundity of wiled populations of *A. lucogaster* where the clutch size was about 4000 eggs (Goulet, 1997).

In *Amblyglyphydodon leucogaster* promiscuous mating by females has been reported and they were capable of laying eggs every second day (Goulet, 1994 ; 1997). Also the spawning usually involved a single male and female, with occasional occurrence of polygynous mating. In all trials in the present study with more than one male observations were similar. A female which spawned with one male deposited eggs in the nest of another male on subsequent spawnings in many occasions. In trials where many functional females were present, continuous egg laying was noted. This pattern of breeding was found in *Neopomacentrus cyanomos*, *N. nemurus*, *N. sindensis* and *P. caeruleus*. Since polygynous mating was noted in *D. carneus* and *P. pavo*, these species may exhibit similar breeding pattern if more number of males were present in the broodstock tanks. Multiple groups were also reported in *Dascyllus marginatus* in large branching coral heads (Fricke, 1980).

Distinct reproductive seasonality is observed for many temperate and subtropical pomacentrids (Richardson *et al.*, 1997 ; Goulet, 1997 ; Moyer

and Bell, 1976).Anemone fishes usually are continuous breeders (Allen, 1972). Non seasonal spawning is seen in *Amphiprion melanopus* (Ross, 1978). Spawning was almost continuous for *A. sebae.* It involved continuous period of active spawning followed by shorter resting periods. From Fig. 1 it is clear that the resting period does not coincide with any particular season of the year. In some temperate and subtropical anemonefishes, the breeding season is in the warmer months of the year (Richardson *et al.*, 1997 ; Moyer and Bell, 1976).

Information regarding the breeding patterns from wild population is not availing for any of the species under study here. But the general spatial and temporal patterns in breeding are not much different from the general pattern of pomacentrids. It is evident from the present study that the three species *Amphiprion sebae, Dascyllus carneus* and *Pomacentrus pavo* are capable of laying eggs round the year with fairly high fecundity. Other species such as *Neopomacentrus cyanomos, N. nemurus* and *Pomacentrus caeruleus* also lay eggs continuously and round the year as observed in other damsel fishes. Such reproductive strategies are advantageous for aquarists to develop the broodstock of these species.

6. EMBRYOLOGICAL STUDIES ON SELECTED POMACENTRIDS

6.1. Introduction

Breeding patterns and behavioural aspects of many tropical and sub tropical pomacentrids have been well documented. Most of the studies on damselfish reproduction are from wild populations. Therefore descriptions of eggs and juveniles are available for relatively fewer pomacentrid fishes and such reports are lacking from Indian waters. Development of broodstock in captivity and *in vitro* studies of eggs is a reliable method for embryological studies.

Description of eggs and embryological studies of certain pomacentrids are available from earlier works. Brinley (1939) studied the development of beaugregory damselfish and Shaw (1955) described the embryonic development of sergeant major damselfish *Abudefduf saxatilis*. Myrberg *et al* (1967) gave an account of the eggs of *Chromis multilineata*. Allen (1972; 1991) described the egg development of Eniwetok anemonefishes. Hoff (1996) studied the egg development of the anemonefish *Amphiprion ocellaris*. Descriptions of the eggs and larvae of *A. saxatilis* were given by Alshuth *et al.* (1998). Other species which were investigated earlier include *Chromis dispilus* (Kingsford, 1985), *Chromis chromis* (Re and Gomes, 1982) and *Abudefduf luridus* (Re, 1980). In the present study, descriptions of eggs and embryonic development of seven species of pomacentrids – *Amphiprion sebae, Pomacentrus caeruleus, Pomacentrus pavo, Neopomacentrus cyanomos, Neopomacentrus nemurus, Neopomacentrus sindensis* and *Dascyllus carneus* – are described with illustrations.

6.2. Materials and Methods

Fertilised egg samples were collected from different spawnings of females of six species, *viz Amphiprion sebae, Neopomacentrus cyanomos, N. nemurus, Pomacentrus caeruleus, Pomacentrus pavo* and *Dascyllus carneus*. The samples were taken on each day till hatching. The length of the egg along the longitudinal axis and the maximum width were measured. The spawning process was carefully observed for all the species. The egg samples collected from the broodstock tanks were brought to the laboratory and maintained in 1 liter glass containers with periodic water exchange. The eggs were continuously observed for studying the early development. The incubation temperature varied from 28 to 29.5° C.

6.3. Results

The eggs of all species were capsule shaped except in *Dascyllus carneus* where it was ovate. The eggs were attached to the substratum at the animal pole. The dimensions of eggs of all species studied are given in Table 1(Page 132). The embryonic development followed a general pattern in all the species studied but there were differences among species in some aspects, especially in the duration to reach different stages.

6.3.1. Embryology of the clownfish *Amphiprion sebae*

1. Fertilised and undivided ovum : Colour of yolk varied from yellow to orange and the blastodisc was present at the animal pole. An eggs 45 minutes after fertilization is shown in Plate 1a.

2. *Two celled ovum* : First cleavage occurred 70 minutes after fertilisation. The blastodisc divided into two blastomeres (Plate 1b). The time of first and subsequent divisions showed slight variations in different batches.

3. *Four celled ovum* : The second cleavage occurred about 85 minutes after fertilisation. The plane of second division was perpendicular to the first division. Four celled ovum is shown in Plate 1c.

4. *Eight celled ovum* : Eight celled ovum was reached after 110 minutes. The plane of the division was parallel to the first cleavage resulting in the formation of two rows of four cells each (Plate 1d).

5. *Sixteen celled ovum* : Sixteen celled stage was reached in about 150 minutes and is shown in Plate 1e. The plane of division was parallel to the second division resulting in the formation of four rows of four cells each.

6. *Blastula* : Blastula stage was formed by the continued horizontal and vertical divisions. Late blastula is reached in about 8 hours, and it is about 10 cells deep forming a cap over the yolk (Plate 2a).

7. *Gastrula* : The germ ring formed by the peripheral band of cells migrated uniformly over the yolk. The germ ring reached beyond half of the yolk in about 16 hours (Plate 2b).

8. *Yolk plug* : The extending germ ring covered almost entire part of the yolk and the small plug extended through the blastopore. This stage was reached in 20 hours (Plate 2c).

9. *Neurula* : The yolk was completely enclosed by the membrane, and the first signs of body form appeared. After about 26 hours small head fold could be seen emerging from the cephalic end of the embryo and two optic vesicles could be noted. Neurula with optic vesicles is shown in Plate 2d. Four or five somites could be seen in the midbody region and the embryo moved from the base of the egg capsule to the center of the egg.

10. *Pigmentation* : Pigmentation started by 36 hours when chromatophores appeared on the yolk as dark spots. About 15 – 16 somites were visible by this

time with the extension of a small tail bud. Due to the appearance of chromatophores the colour of the egg turned dark from yellow or orange and the egg clutch appeared grey in colour; 37 hour old embryo is shown in Plate 2e.

11. Motility : As development proceeded, the movement of the tail had started. The embryo was capable of rotating itself within the egg capsule, and by the third day morning the embryo reversed the position and it was facing towards the distal end of the egg capsule; 50 hour old embryo is shown in Plate 3a.

12. Fourth day embryo : By the fourth day there was distinct development of the cephalic region and heart beat was visible. Pigmentation on the eyes also started. The size of yolk sac decreased with development. 98 hour old embryo is shown in Plate 3b.

13. Fifth day embryo : Pigmentation in the eyes became dense and the eyes were opaque. Melanophores on the yolk sac were dendritic. Melanophores were also present on the trunk. 122 hour old embryo is shown in Plate 3c.

14. Sixth day embryo : The embryo had considerably grown in size and almost filled the egg capsule. The jaws were mobile; 146 hour old embryo is shown in Plate 3d.

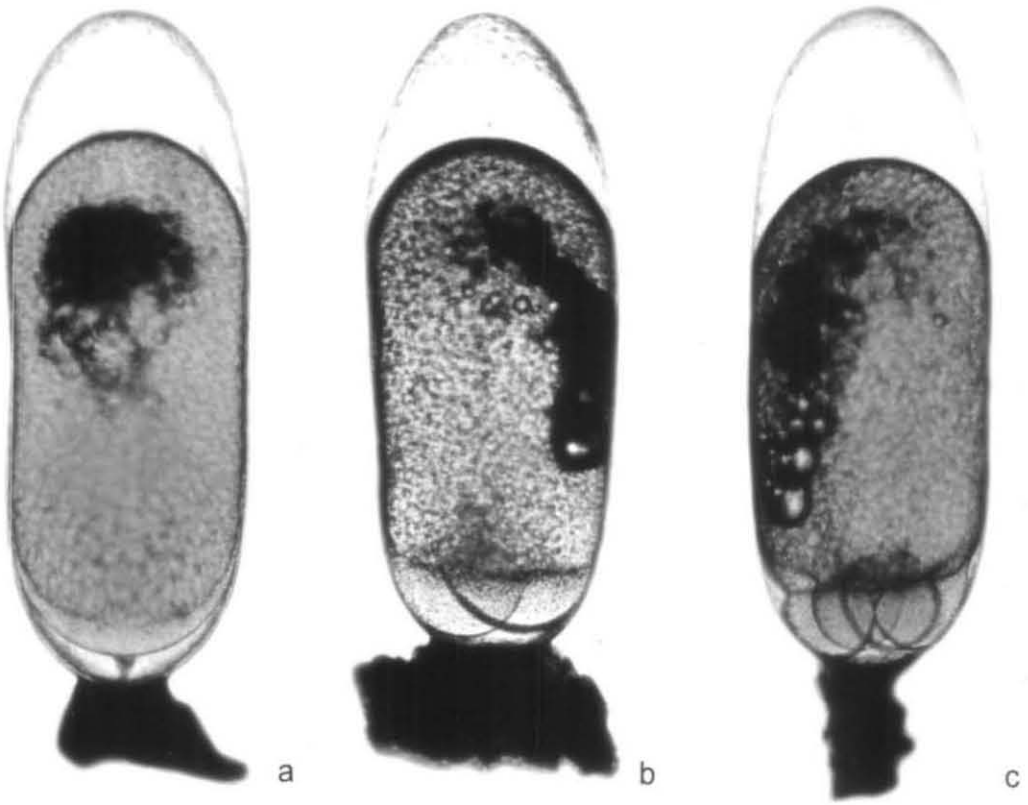
15. Fully developed embryo : Yolk had reduced considerably. Silvery glittering colour of eyes developed. Hatching took place usually on the seventh or eighth day after sunset; 174 hour old embryo is shown in Plate 3e.

6.3.2. Embryology of damselfishes *Pomacentrus* spp. and *Neopomacentrus* spp.

The duration of development were identical for *Pomacentrus caeruleus*, *P.pavo*, *Neopomacentrus cyanomos*, *N. nemurus* and *N. sindensis*. The developmental stages of these species are described with illustrations of *P. caeruleus* and *N. cyanomos* eggs.

Plate 1. Embryonic developmental stages of *A. sebae*

Average Length 2.06 mm



a

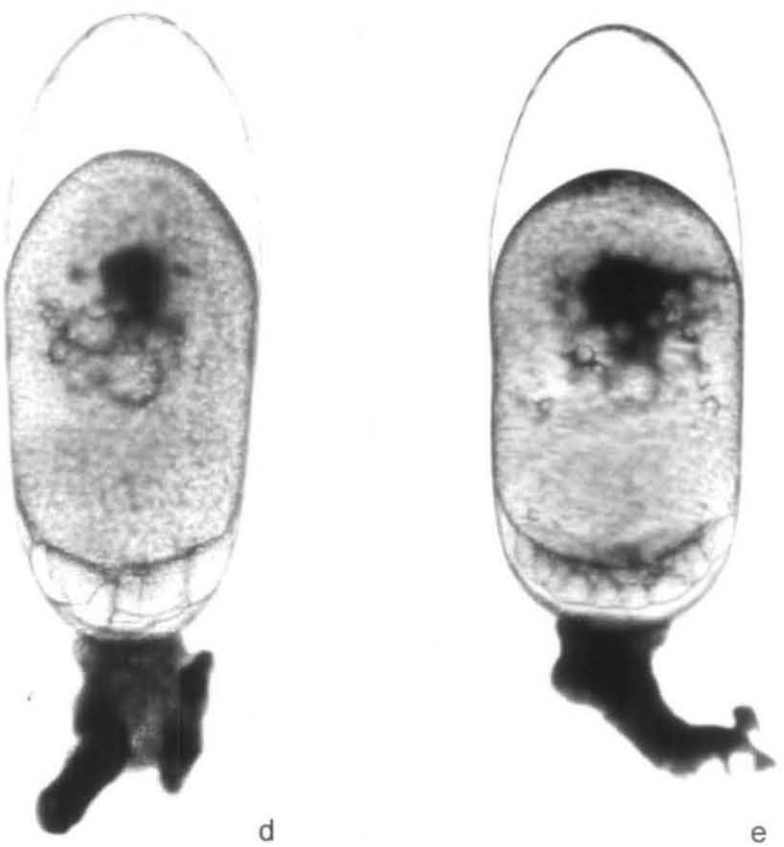
Fertilised and undivided ovum

b

Two celled stage

c

Four celled stage



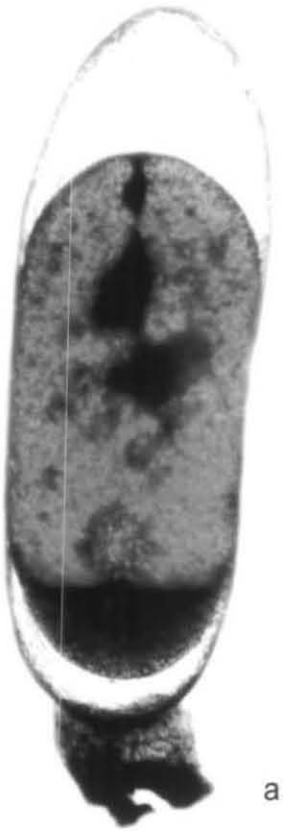
d

Eight celled stage

e

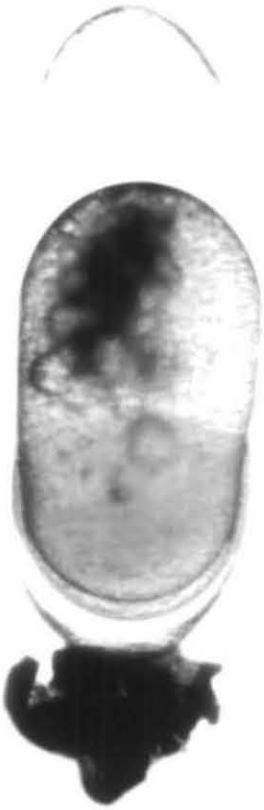
Sixteen celled stage

Plate 2. Embryonic developmental stages of *A. sebae*



a

Blastula

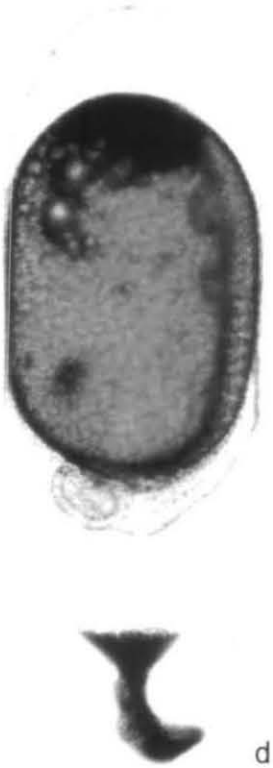


b

Gastrula

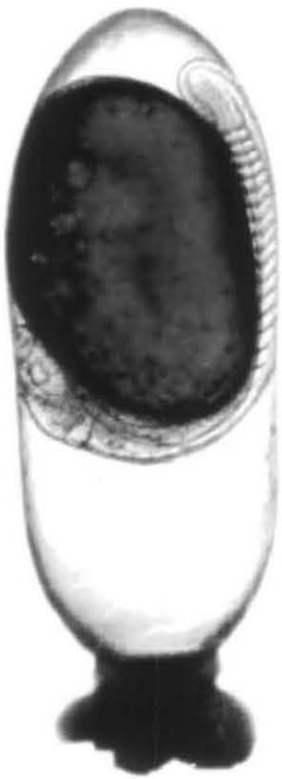
c

Yolk Plug



d

Neurula



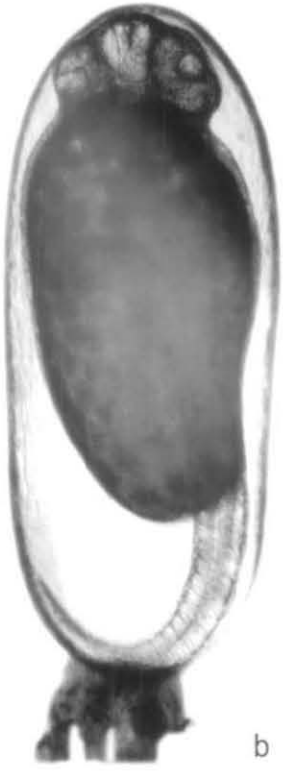
e

Pigmentation

Plate 3. Embryonic developmental stages of *A. sebae*

a

Motility



b

Fourth day embryo

c

Fifth day embryo

d

Sixth day embryo

e

Fully developed embryo

1. *Fertilised and undivided ovum* : The undivided ovum contained a blastodisc at the animal pole and a large yolky region with one large oil globule and a few smaller ones. *P. caeruleus* eggs 60 minutes after fertilisation is shown in Plate 4a.

2. *Two celled ovum* : The first cleavage occurred in 75 to 85 minutes after fertilisation for all the species. *P. caeruleus* egg 90 minutes after fertilisation is shown in Plate 4b.

3. *Four celled ovum* : The second cleavage occurred in 95 – 105 minutes after fertilisation. The plane of second cleavage was at right angles to the first cleavage. A 110 minute old *P. caeruleus* egg is shown in Plate 4c.

4. *Eight celled ovum* : Eight celled stage was reached in 120 – 130 minutes after fertilisation. Eight celled ovum of *P. caeruleus* is shown in Plate 4d.

5. *Thirty two celled ovum* : This stage was reached in 180 – 190 minutes and the plane of the division was parallel to the first cleavage and four furrows were formed. This resulted in the 32 cell stage with four rows of eight cells each. *P. caeruleus* egg 180 minutes after fertilisation is shown in Plate 4e.

6. *Blastula* : The continued horizontal and vertical divisions resulted in the formation of blastula. After eight hours, the blastula was a dense cell mass of about 10 cells deep forming a cap over the yolk. Eight hour old blastula of *P. caeruleus* is shown in Plate 4f and that of *N. cyanomos* in Plate 6a.

7. *Gastrula* : The peripheral layer of cells migrated over the yolk and formed a germ ring. The germ ring advanced over the yolk towards the distal end and after about 14 hours it reached about one third of the yolk; 14 hour old gastrula of *P. caeruleus* is shown in Plate 4g.

8. *Yolk plug* : This is the stage at which most part of the yolk is enclosed within the germ ring leaving a small yolk plug outside. This stage was reached at about 20 hours for all species. Yolk plug stage of *P. caeruleus* is shown in Plate 5a.

9. Neurula : The yolk was completely covered by the membrane. This took about 22 hours and the neurula stage of *N. cyanomos* is shown in Plate 6b. After 26 hours a small head fold lifted from the cephalic end and two optic vesicles were visible. Neurula with optic vesicles of *P. caeruleus* is shown in Plate 5b and that of N. *cyanomos* is shown in Plate 6c.

10. Motility : The length of the embryo increased considerably and it was possible for it to move the tail at about 32 hours. The embryo was capable of rotating within the egg capsule. 30 hour old of *N. cyanomos* is shown in Plate 6d and 32 hour old embryo of *P. caeruleus* is shown in Plate 5c. After 38 hours the head was well developed and the embryos were facing the distal end of the egg capsule. Heart beat was visible at this time. 38 hour old egg of *P. caeruleus* is shown in Plate 5d.

11. Third day egg : Pigmentation started, melanophores appeared on the trunk and the length of the embryo increased about two fold. 50 hour old embryo of *P. caeruleus* is shown in Plate 5e and that of *N. cyanomos* is shown in Plate 6e.

12. Fourth day egg : The embryo completely filled the egg capsule and the yolk content had reduced. Dense pigmentation of retina and the eyes became opaque. Jaws were movable. 74 hour old embryo of *P. caeruleus* is shown in Plate 5f and that of *N. cyanomos* in Plate 6f. The egg usually hatched in 84 to 86 hours.

6.3.3. Embryology of *Dascyllus carneus*

The egg size and duration of development of *D. carneus* were different from the other species studied.

1. Fertilised and undivided ovum : The blastodisc could be seen extending from the yolky region towards the animal pole. There was a single large oil globule (Plate 7a).

Plate 4. Embryonic developmental stages of *P. caeruleus*

a

Fertilised and undivided ovum

b

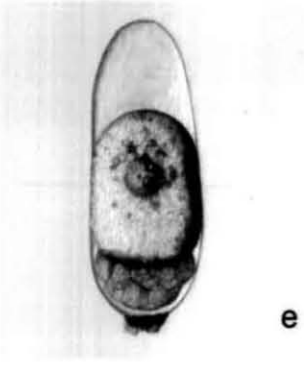
Two celled stage

c

Four celled stage

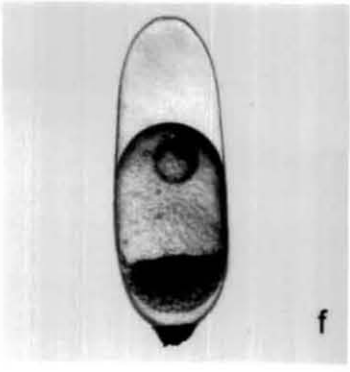
d

Eight celled stage



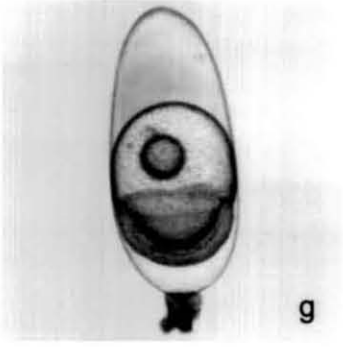
e

32 celled stage



f

Blastula



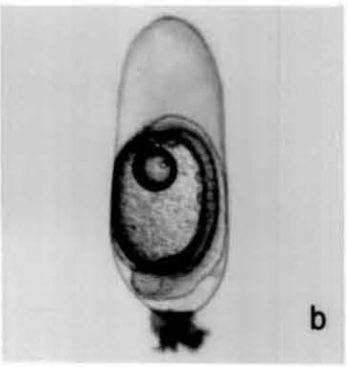
g

Gastrula

Plate 5. Embryonic developmental stages of *P.caeruleus*

a

Yolk Plug

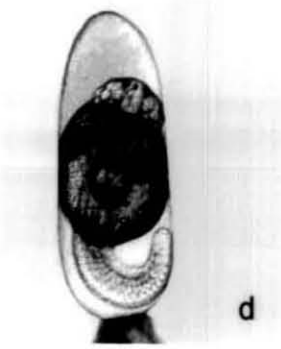


b

Neurula

c

Motility



d

38 hour old egg

e

Third day egg

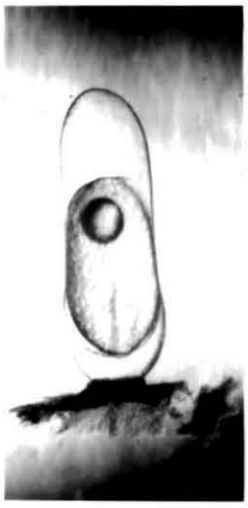
f

Fourth day egg

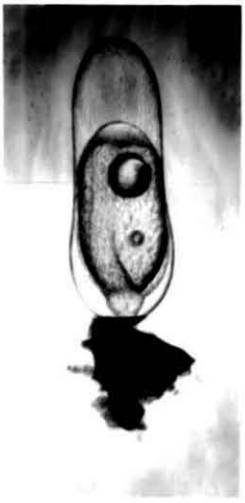
Plate 6. Embryonic developmental stages of *N. cyanomos*

Average Length 1.15 mm

a. Blastula



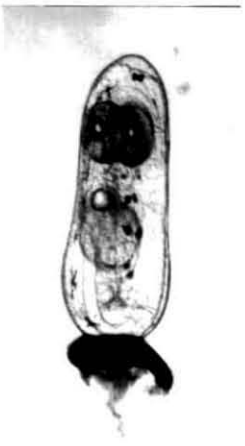
b. Neurula



c. Optic vescicles

d. 30 hr old egg

e. Third day egg



f. Fourth day egg

2. *Two celled ovum* : First cleavage occurred after 60 minutes when two blastomeres were formed from the blastodisc(Plate 7b)

3. *Four celled ovum* : Second cleavage was noted after 70 minutes. The plane of second division was at right angles to the first cleavage (Plate 7c).

4. *Eight celled ovum* : Eight celled ovum was formed after 85 minutes and is shown in Plate 7d.

5. *Sixteen celled ovum* : This stage was formed by the double cleavage in the plane parallel to the second cleavage resulting in the formation of four rows of four cells each. It was formed in 100 minutes (Plate 7e).

6. *Blastula* : Formed by the repeated vertical and horizontal divisions. Early blastula was formed by about 160 minutes when the cell mass was about 5 cells deep (Plate 7f). Late blastula was reached by 3.5 hours when the cell mass was about 10 cells deep.

7. *Gastrula* : The germinal ring formed by the peripheral layer of cells uniformly migrated over the yolk. It reached to about one third of the yolk by eight hours (Plate 7g).

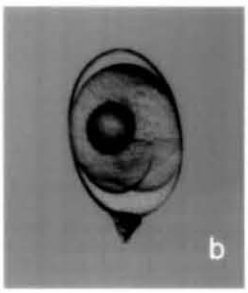
8. *Second day egg* : After 26 hours the head and trunk began to differentiate and the embryo was capable of moving the tail. Yolk content reduced considerably. 26 hour old egg is shown in Plate 7h. By the evening of the second day a well differentiated head was developed with visible optic vesicles. Melanophores appeared on the trunk and the embryo was capable of rotating within the egg capsule. 34 hour old egg is shown in Plate 7i.

9. *Third day egg* : The embryo was fully grown and occupying the entire egg capsule. The embryo rested in the egg with three body folds. The jaws were not movable and eyes were not pigmented. 54 hour old embryo is shown in Plate 7j. The eggs hatched in 58 – 60 hours.

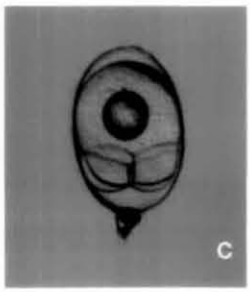
Plate 7. Embryonic developmental stages of *D. carneus*

Aveage Length 0.67 mm

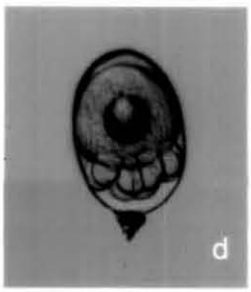
Fertilised and undivided ovum



Two celled stage



Four celled stage



Eight celled stage

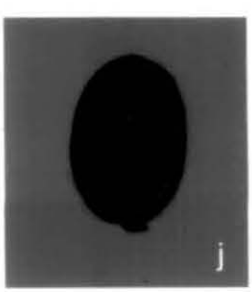
Sixteen celled stage

Blastula

Gastrula

26 hour old egg

34 hour old egg



Third day egg

Table 1 : Egg dimensions of seven pomacentrid fishes

Species	Numbers measured	Length	Max. width
A. sebae	129	2.06 ± 0.0059	0.79 ± 0.0032
D. carneus	88	0.67 ± 0.002	0.45 ± 0.0015
N. cyanomos	137	1.15 ± 0.0024	0.41 ± 0.0019
N. nemurus	144	1.08 ± 0.003	0.41 ± 0.002
N. sindensis	104	1.15 ± 0.0035	0.43 ± 0.0025
P. caeruleus	93	1.02 ± 0.0041	0.40 ± 0.0028
P. pavo	83	1.14 ± 0.0033	0.45 ± 0.0025

6.4. Discussion

Pomacentrids lay demersal eggs attached to submerged objects. The shape of eggs range from ovate to capsule shaped in different species (Allen,1972). All the species in the present study conformed to this general pattern. *D. carneus* eggs were ovate and in others they were capsule shaped. The eggs of *Amphiprion chrysopterus* was 2.4 × 0.9 mm (Allen, 1972) and Hoff (1996) reported the lengths of anemonefish eggs to range from 2.0 to 2.4 mm. The normal size of *A. sebae* eggs in the present study was within this range. Eggs of *Pomacentrus* and *Neopomacentrus* species were identical having lengths slightly above 1mm and breadth about 0.4 mm. Allen (1972) reported that *Chromis* and *Dascyllus* eggs were generally small and ovoid and are about 0.6 × 0.4 mm. The size of *D. carneus* eggs was comparable to the above observation.

For studying the embryology, eggs initially laid were taken in all cases. This is because the spawning activity lasted for one or two hours and the eggs were laid by the females randomly over the whole area of the egg clutch. Usually the male fertilizes the eggs immediately after they are laid by the female and hence the time of fertilization varies within the clutch. Similar observations were recorded for wild populations of *A. chrysopterus* (Allen, 1972). Therefore when spawning is completed, the clutch contains eggs which are fertilised at different times and they will be present at random. So when samples are taken after spawning, it will be difficult to ascertain the exact time of fertilisation. Shaw (1955) studied the embryology of *Abudefduf saxatilis* after artificial fertilisation by stripping eggs and pouring sperm suspension prepared by macerating the testis over it.

The embryonic development of all the species studied conformed to the general pattern given by Shaw (1955) and Allen (1972, 1991). The duration

of development was shorter in smaller eggs. The early development in the present study was faster than those reported by Shaw (1955) even for larger eggs of clownfish. This may be due to the difference in incubation temperature which was 24° C in the latter case. Temperature, water quality and to some extent photoperiod affect the embryonic development (Hoff, 1996). Slower development and delayed hatching was noted on some occasions in all species except *D. carneus,* mostly at times of lower temperatures.

7. LARVAL REARING AND LARVAL DEVELOPMENT OF SELECTED POMACENTRIDS

7.1. Introduction

Larval rearing of marine finfishes is relatively complicated due to the small size of larvae, feeding problem and peculiar water conditions required in the larval rearing system. As a result, only very few species have been reared in captivity with good survival rates. Among marine ornamental fishes reared in captivity, the maximum number is contributed by the family pomacentridae. About 20 species of pomacentrids have been reared in captivity the world over (Arvedlund *et al.*, 2000), and in India there are two reports of successful larval rearing of anemonefishes (Gopakumar *et al.*, 1999 ; Ignatius *et al.*, 2001). Though anemonefishes have been reared in various parts of the world with good survival rates, the survival rates were low in damselfishes in most attempts. This is mainly due to the smaller size and longer larval duration of damselfishes when compared to anemonefishes.

In recent years much attention has been paid to the studies on larval behaviour, biology and larval rearing of marine fin fishes. Most of such attempts were on larger fishes of food value. However, there are many reports on the larval biology and larval rearing of many ornamental fishes including pomacentrids. Pothoff *et al.* (1987) described the osteological development of larvae and juveniles of the yellow tail damsel fish *Microspathodon chrysurus*. Growth and identification of pomacentrid larvae from East Pacific was described by Victor (1987). Thresher *et al.* (1989) estimated the duration of larval stage in Pacific damselfish and reported that the mean planktonic duration varied between 0 and 37.4 days. Wellington and Victor (1989) estimated the larval duration of 100 species of Pacific and Atlantic damselfishes from daily growth

increments on otolith of juveniles. Larval duration and growth rates before and after settlement of two species of damselfishes, *Pomacentrus coelestis* and *Chromis atripectoralis,* were investigated by Thorrold and Milicich (1990). They found out that the post settlement growth rates were faster than the pre settlement growth for both the species. The size and levels of yolk reserves of the newly hatched larvae are significantly influenced by food availability, social interaction and size of females (Kerrigan, 1997). McCormick (1998) reported that the maternal levels of the stress hormone cortisol is responsible for the effect on larval morphology and yolk size. The maternally derived cortisol and testosterone are also important in regulating growth, development and nutritional resources of the larvae of *Pomacentrus amboinensis,* and therefore, factors that affect the maternal cortisol and testosterone levels may have a major impact on larval mortality schedules (McCormick, 1999). Larval development of the laboratory reared sergeant major *Abudefduf saxatilis* was described by Alshuth *et al.* (1998). The eggs and larvae of 52 species of pomacentrids under tank breeding conditions were reported by Tanaka (1998).

There are many reports of successful raring of various species of anemone fishes from different parts of the world (Alava and Gomes, 1989 ; Allen, 1991 ; Hoff, 1996 ; Wilkerson, 1998 ; Gopakumar *et al.*, 1999 ; Ignatius *et al.*, 2001). However, there are fewer reports on successful rearing of damsel fishes (Pothoff *et al.*, 1987 ; Danilowicz and Brown, 1992 ; Moe, 1992 ; Job *et al.*, 1997 ; Alshuth *et al.*, 1998). A great deal of experimental works have also been conducted on various aspects of larval rearing of clownfishes. Frakes and Hoff (1982) studied the effect of high nitrate – N on the growth and survival of juveniles and larvae of *A. ocellaris.* Alayse (1984) reported increased survival rate by giving enriched feed to *A. ocellaris.* Coughlin *et al.* (1992) studied the feeding behaviour and searching pattern of *Amphiprion perideraion* larvae. Coughlin (1993) reported the search space of the same species to be 0 – 120° from long axis in the horizontal plane and 0 – 150° in the transverse plane. The effect of larval feeding on the body condition of *A. melanopus* was described by Green and McCormick (1999). Arvedlund *et al.* (2000) studied the effect of

photoperiod in the larval rearing of *Amphiprion melanopus* and reported a 16 hour light and 8 hour dark light regime to be ideal for the species. Green and McCormick (2001) reported that *A. melanopus* hatched with highly differentiated digestive tract and has the ability to capture and ingest prey items. It was also reported that the alimentary tract changes rapidly throughout the larval period.

In the present study larval rearing was attempted for one species of anemonefish, *Amphiprion sebae* and four species of damselfishes, *Neopomacentrus cyanomos, N. nemurus , Pomacentrus caeruleus* and *P. pavo.* Larval development was studied for one species of anemone fish *A. sebae* and three species of damsel fishes, *N. cyanomos, P. caeruleus* and *P. pavo.*

7.2. Material and methods

Larval rearing was done for one species of anemonefish *Amphiprion sebae* and four species of damselfishes *viz. Neopomacentrus cyanomos, N. nemurus, P. caeruleus* and *P. pavo.* The details about the brood stock of all the species is given in chapter 4. Egg of all the species were allowed to hatch in the parental tanks. The newly hatched larvae were collected along with water in small plastic buckets. The larvae were counted and introduced into larval rearing tanks.

Rectangular FRP tanks of 250 litre capacity were used for rearing anemonefish larvae. A closed continuous flow through system was provided in the larval rearing tanks. The flow rate was maintained at 15 to 20 litre / hour. The outlet pipe was so arranged that the larvae were not sucked by it. The inner end of the outer pipe was covered with nets of 100 µ mesh to prevent the loss of rotifers. The net was cleaned every day and changed periodically. The

temperature of the water during the study period ranged between25°C and 31°C, pH 7.78 to 8.09, salinity 28 – 34 ppt, nitrate 0.17 - 0.94 ppm, nitrite 0.024 – 0.176 ppm, and ammonia 0.01 – 0.09 ppm. The newly hatched larvae were fed with the rotifer *Brachionus rotundiformes* (Lorica length range 150 to 180 μ) five times daily. The concentration of rotifer was 4 to 7 numbers / ml immediately after feeding. There was no feeding between 06. 00 pm and 07. 00 am. After four days freshly hatched *Artemia* nauplii were also given along with rotifers. The larvae were fed with *Moina micrura* after tenth day. The detailed feeding schedule is given in Table 1. The bottom and sides of the tanks were cleaned everyday. Lighting was provided for the larval tanks for 16 hours a day from tube lights kept 2 m above the larval tanks. Twelve experimental trials were done from larvae of three different breeding pairs. Two larvae were sampled on alternate days from three different trials to study the development. The morphometric measurements and development were studied as in Tucker and Alshuth (1997) and Alshuth *et al.* (1998).

For rearing the larvae of damselfishes rectangular RCC tanks of one tonne capacity were used. Direct light was avoided in the larval tanks and were provided with diffused daylight by providing transparent sheet roof and in nights from a 25 W bulb hung 3 m above the tanks. Thus about 12 hours light was given eveyrday. Water temperature range was 24 – 30.5°, Salinity, 28 – 34 ppt , pH 7.51 – 7.94, Nitrate 0.21 – 1.03 ppm nitrite, 0.028 – 0.49 ppm and ammonia 0.023 - 0.088.ppm.Green water system was adopted for the rearing of damsel fish larvae. Pure cultures of microalga *Chlorella* was added to the tank to green up the sea water. The concentration of the green algae was maintained at 0.1 – 0.2 million cells / a ml. Fresh culture of Chlorella was added to the tank every day to compensate the loss due to water exchange. Oil skimmers were setup in the tank to remove the oil film formed on the water surface. These were fabricated with slight modification from that described in Lim (1993). The skimmer consisted of a triangular frame made of 0.5 " PVC pipe fitted with a nozzle at one side. Airflow was provided through the nozzle

Table 1 . Feeding schedule for rearing *A. sebae* larvae

Days after hatching	Feed	Concentration	Frequency / day
0 – 4	Rotifer	4.0 – 7.0 nos / ml	5
4 – 10	Rotifer	4.0 – 7.0 nos / ml	5
	+		
	Artemia nauplii	1.0 – 1.5 nos / ml	3
10 - 15	*Artemia* nauplii	1.0 – 1.5 nos / ml	3
	+	0.5 / ml approx.	4
	Moina		

Table 2 . Feeding schedule and scheme of water exchange for rearing damsel fish larvae

Days after hatching	Feed type	Conc.	Frequency / day	Water exchange / day
0 – 3	No feed Accepted ciliates			50% in two times
4 – 10	Boiled & Smashed mussel meat sieved through 75 µ net		5	"
10 – 15	Boiled and smashed mussel meat sieved through 120 µ net		5	80% in three times
15 – 23	"		5	100% in four times
	Artemia nauplii	0.5-1.0/ml	3	
23 – till metamorphosis	*Artemia* nauplii	1.0 / ml	3	"
	Moina	0.5 / ml	3	

towards the inner area of the frame. The oil film and other dust particles were sucked by the gentle water flow created due to the movement of air. In the present trials a covering with bolting cloth was given at the lower side of the skimmer touching the water surface to prevent the larvae getting trapped in the accumulated oil film. The rearing medium contained ciliates developed from the micro algal debris and rotifer *Brachionus rotundiformes* at a concentration of 2 – 3 numbers / ml. The early larvae were found to feed on the former for the first one week. From fourth day onwards finely smashed ovaries and tissues of the mussel *Perna indica* filtered through 75 µ bolting silk was given four times daily. It contained mussel eggs and smaller tissue fragments. The particle size was increased after 10 days by filtering through 120 µ bolting silk. From 15th day onwards freshly hatched *Artemia* nauplii was given thrice daily. After three weeks most of the larvae were capable of feeding *Moina micrura* and were fed four times a day till metamorphosis. Water exchange was done in the morning and evening @ 50% initially, and increasing to 100% later. Details of water exchange and feeding schedule is given in Table 2. Two larvae of each species were collected on alternate days to study the morphometry and development. The dead larvae on days of mortality were also used for the study. Morphometry and development were noted as in Tucker and Alshuth (1997) and Alshuth *et al.* (1998). Six morphometric measurements *viz.* total length, body length, head length, head depth, body depth and eye diameter were taken with an ocular micrometer under a monocular microscope with an accuracy upto one hundredth of a millimeter. Morphometric ratios were calculated as percentage of body length. Body length refers to notochord length till flexion, and to standard length in post flexion stages.

7.3. Results

7.3.1. Larval rearing

The hatchlings of the five species were pelagic with a single finfold and movable jaws. Complete yolk exhaustion took place in 2 – 3 days for all the species. The total length and maximum mouth gape of the hatchlings of the five species are given in Table 3.

7.3.1.1. Anemonefish

Twelve experimental trials were made for rearing the anemonefish larvae. The larvae metamorphosed and the first white bar across the operculum appeared in 11 – 16 days. The mean survival after 15 days was 35.4% and ranged from 6.6% to 74.6%. Eventhough there was wide variation in the duration of metamorphosis among different batches, the variation within a batch was very less. Once started, all individuals metamorphosed in 1 or 2 days. The mean length of larvae / juvenile is plotted in Fig. 1. Percentage survival of the twelve trials taken at two days interval till metamorphosis is shown in Fig. 2. The survival rate after first three days averaged 74.08% and ranged from 47 – 94%. The rapid declining trend continued till 7^{th} day and the mortality was less thereafter. The larvae were pelagic in the first week and they moved towards the bottom as they approached metamorphosis. Metamorphosed larvae were introduced in to the sea anemone *S. haddoni* and they quickly acclimated to it.

7.3.1.2. Damselfishes

Two trials each were successful for the four damselfish species. The survival rates were very low compared to anemonefish and ranged from 0.1% to 5.4% for *N. cyanomos* (Fig. 3), 0.6% to 2.1% for *N. nemurus* (Fig. 4) and 2.0% to 6.0% for *P. caeruleus* (Fig. 5). For *P. pavo*, only experimental

Table 3: Average total length and maximum mouth gape of hatchlings of the five species

Species	Total length (mm)	Mouth opening (µm)
A. sebae	3.97	380
N. cyanomos	1.82	158
N. nemurus	1.72	109
P. caeruleus	2.31	181
P. pavo	2.08	189

Fig.1 Average length of larvae / juvenile of different species of pomacentrids during the rearing period

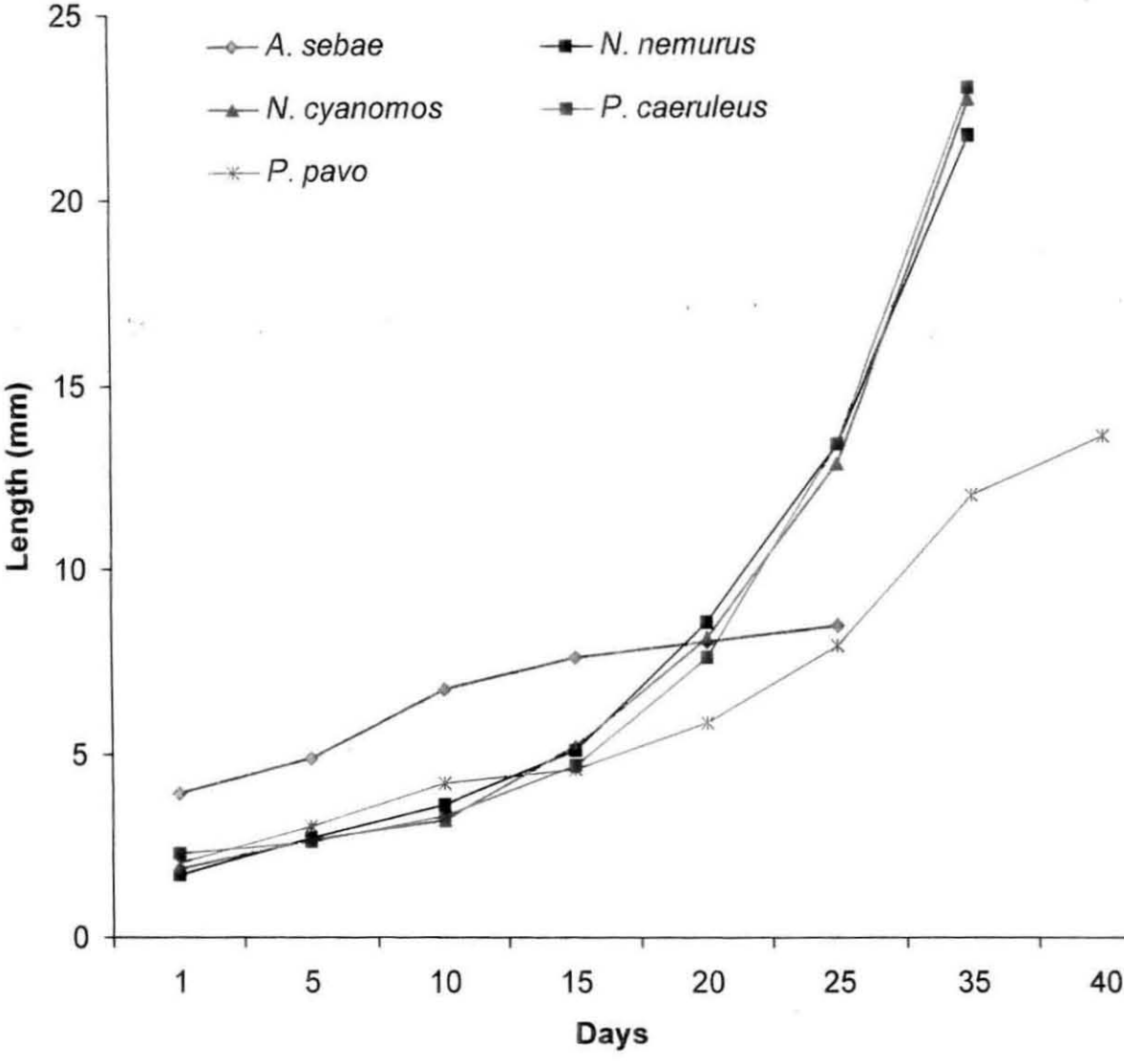


Fig 2. Average survival in 12 trials of *A. sebae* in percentage

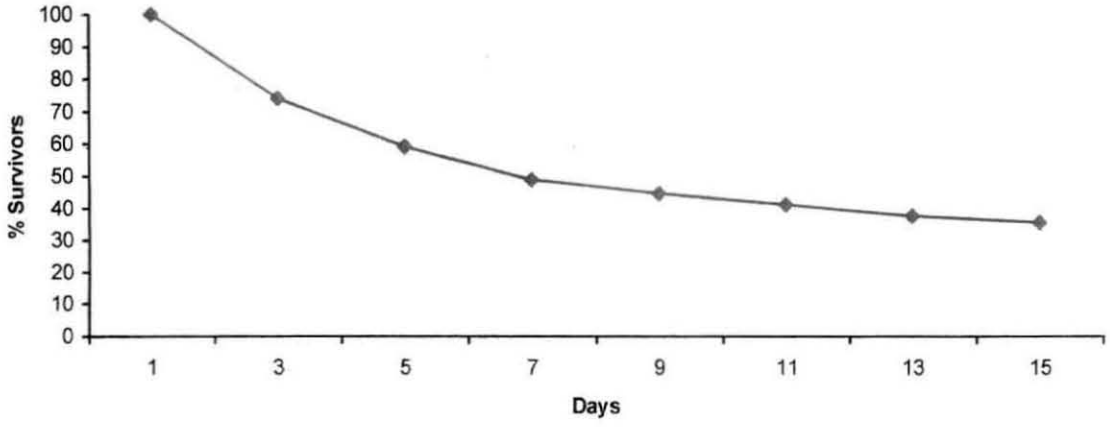


Fig. 3. Percentage survival in two larval rearing trials of *N. cyanomos*

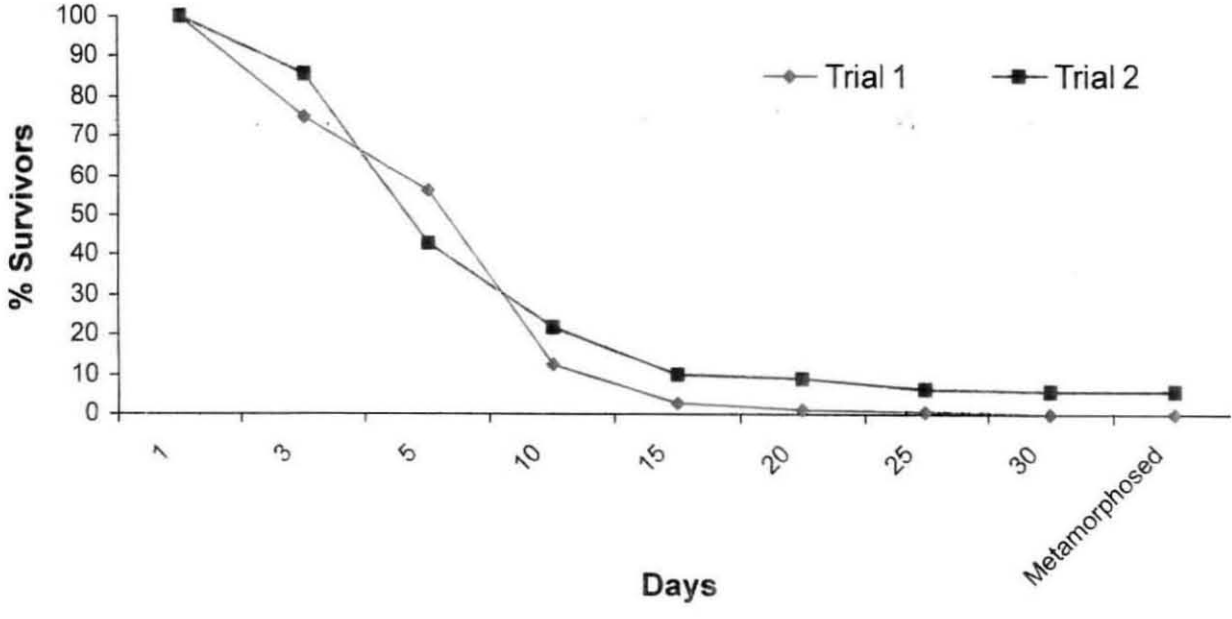


Fig. 4. Percentage survival in two larval rearing trials of *N.nemurus*

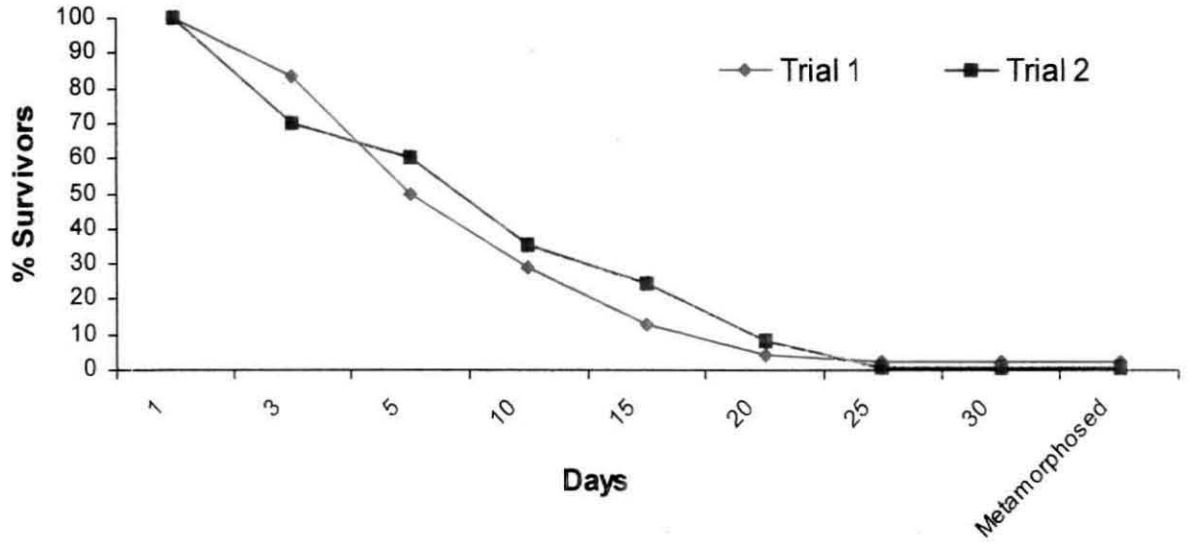
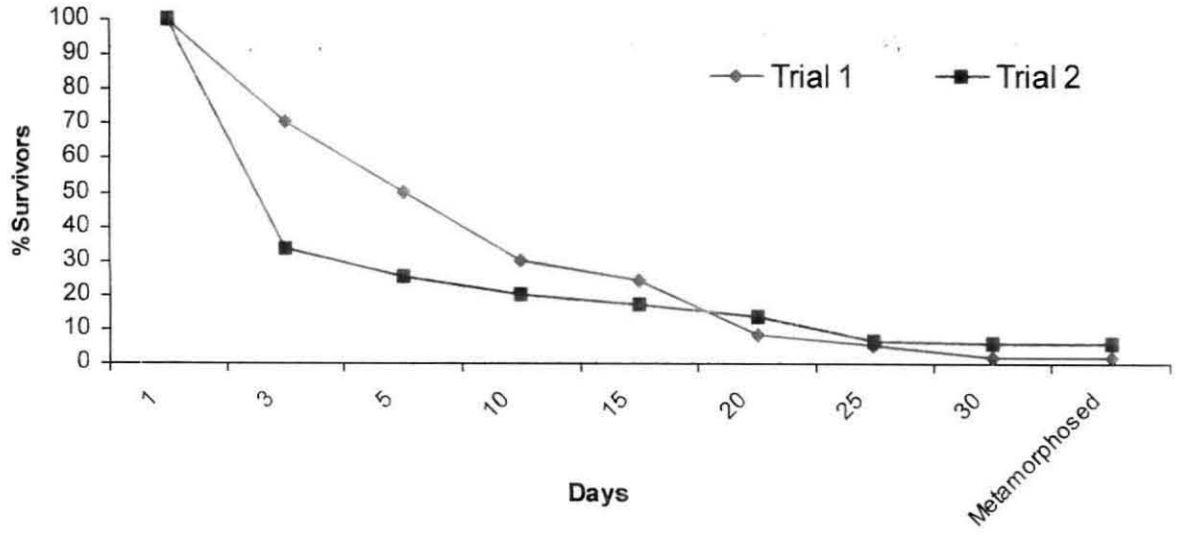


Fig. 5. Percentage survival in two larval rearing trials of *P.caeruleus*



success was achieved, and two and three numbers of larvae respectively metamorphosed in the first and second trials (Fig. 6). There was a 15 to 30% loss in the initial two days for the damselfishes except in trial 2 for *P. caeruleus* where 67% was lost initially. The duration of metamorphosis was 29 to 33 days for *N. cyanomos, N. nemurus* and *P. caeruleus* whereas it was 37 to 41 days for *P. pavo.* Metamorphosis of all the individuals in a batch occurred within 5 to 10 days after start of the appearance of colour in the first individual. Juveniles of the four species became benthic as they approached metamorphosis. The newly metamorphosed juveniles established territories around coral pieces and small earthen pots placed in the growout tanks and they defended the territory. Mean length of the larvae at five day intervals till metamorphosis is given in Fig.1.

7.3.2. Larval development

Morphometry of the larvae of all the species changed after initiation of flexion. The development of larvae of the four species are described below.

7.3.2.1. Anemonefish

Morphometric measurements of *Amphiprion sebae* larvae at different stages is given in Table 4 and ratios with respect to body length is given in Table 5.

i. *Hatchlings* :- The newly hatched larvae had an average length of 3.97mm and the length ranged from 3:70 to 4.25mm. There was a single finfold without any fin rays. Maximum mouth opening ranged from 360 to 400 µm (Plate 1a).

Fig. 6. Percentage survival in two larval rearing trials of *P. pavo*

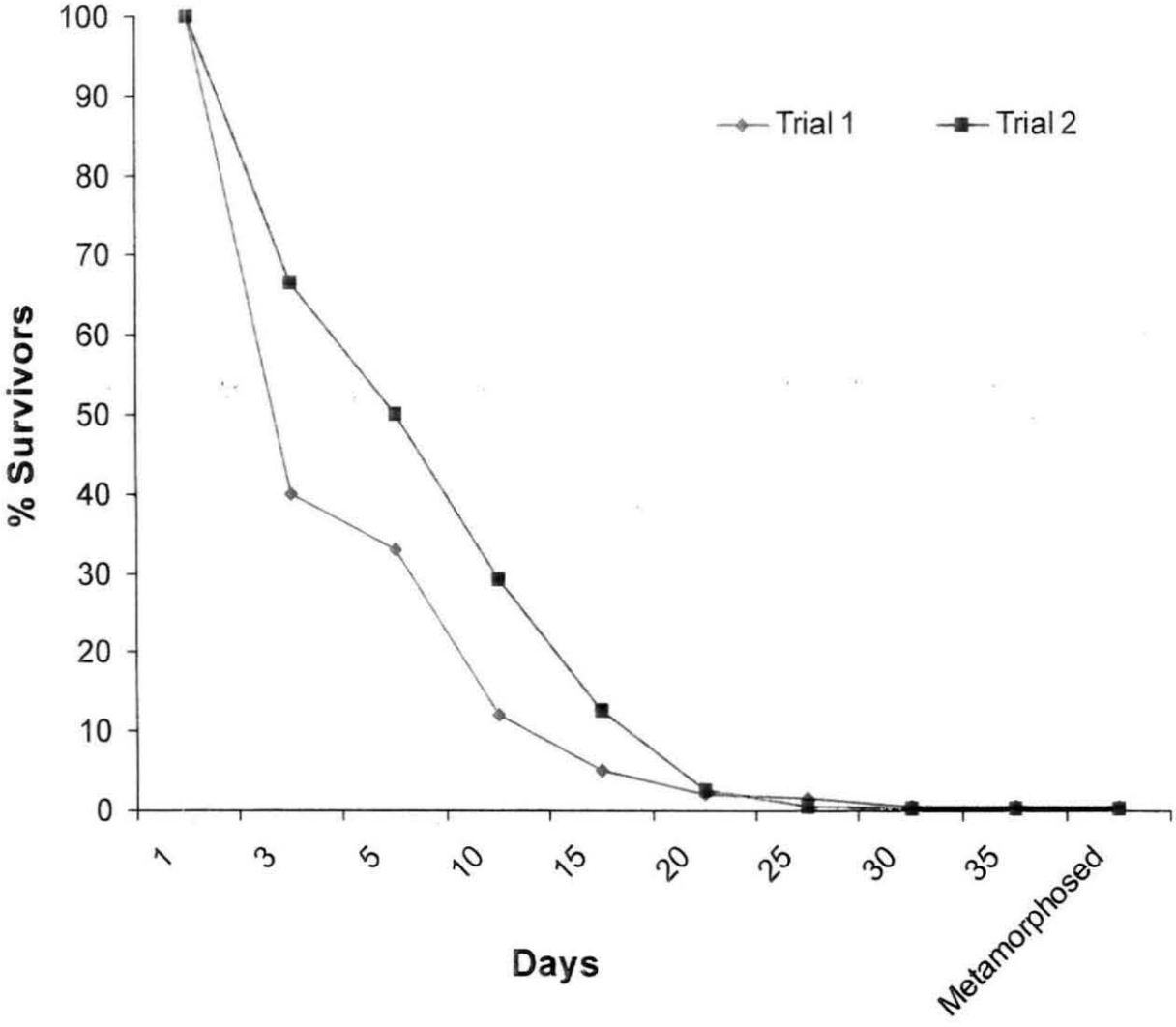


Table 4 . Morphometry of larvae of *A. sebae* during different stages of growth
Mean ± standard deviation in the first line and range in the second line

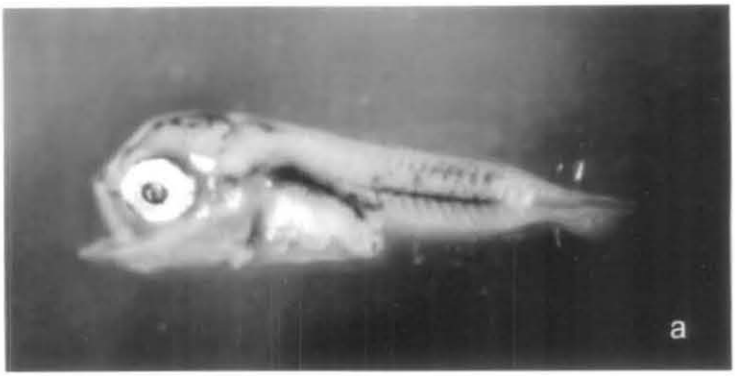
Measurements (mm)	Hatchlings	Pre flexion	Flexion	Post flexion	Early juvenile
Total length	3.97 ± 0.19 3.70 – 4.25	4.91±0.15 4.66–5.09	5.70 ± 0.27 5.46 – 6.16	6.75 ± 0.40 6.23 – 7.15	7.63 ± 0.38 7.26 –8.07
Body length	3.83 ± 0.18 3.56 – 4.11	4.63 ± 0.09 4.47 – 4.73	4.98 ± 0.27 4.80 – 5.46	5.32 ± 0.20 5.06 – 5.50	5.84 ± 0.21 5.57 – 6.23
Head length	0.95 ± 0.076 0.88 – 1.14	1.15 ± 0.11 1.43 – 1.76	4.75 ± 0.08 1.67 – 1.87	1.91 ± 0.14 1.72 – 2.05	2.26 ± 0.21 2.09 – 2.57
Head depth	0.99 ± 0.06 0.92 – 1.06	1.39 ± 0.15 1.10 – 1.50	1.61 ± 0.09 1.54 – 1.76	1.88 ± 0.17 1.65 – 2.05	2.17 ± 0.25 1.98 – 2.53
Body depth	0.94 ± 0.038 0.88 – 0.99	1.35 ± 0.15 1.06 – 1.47	1.69 ± 0.10 1.58 – 1.83	1.86 ± 0.11 1.69 – 1.98	2.24 ± 0.21 2.05 – 2.57
Eye diameter	0.51 ± 0.023 0.48 – 0.55	0.56 ± 0.02 0.55 – 0.59	0.63 ± 0.03 0.62 – 0.69	0.76 ± 0.06 0.70 – 0.81	0.86 ± 0.06 0.81 – 0.92
Number or specimen	10	6	5	5	5

Table 5 . Morphometric ratios of larvae of *A. sebae* expressed as percentage to body length

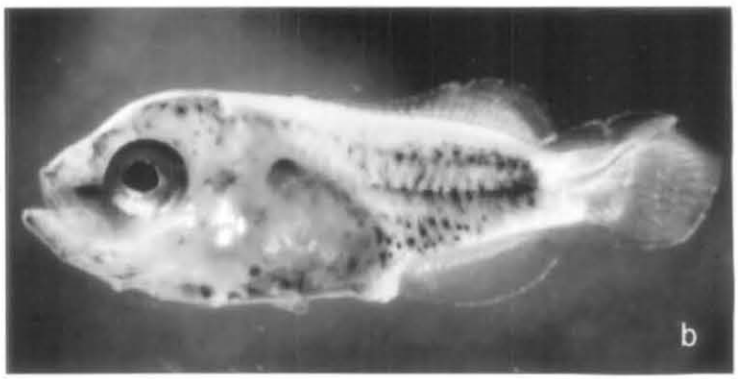
Mean ± standard deviation in the first line and range in the second line

Measurements (mm)	Hatchlings	Pre flexion	Flexion	Post flexion	Early juvenile
Total length	103.77 ± 0.60 102.57 – 104.85	105.88 ± 1.32 104.25 – 107.61	114.63 ± 1.86 112.82 – 117.21	126.81 ± 3.04 123.12 – 130.00	130.79 ± 1.21 129.53 – 132.73
Head length	24.84 ± 1.55 23.65 – 29.08	33.53 ± 3.01 30.69 – 39.37	35.14 ± 0.69 34.25 – 36.07	35.22 ± 1.39 33.99 – 37.27	38.78 ± 1.72 37.50 – 41.25
Head depth	25.87 ± 1.20 23.81 – 28.06	30.04 ± 2.77 24.61 – 31.82	32.42 ± 0.38 32.08 – 32.99	35.39 ± 1.86 32.61 – 37.27	37.13 ± 2.24 35.55 – 40.61
Body depth	24.51 ± 0.63 23.65 – 25.40	29.1 ± 2.74 23.71 – 31.08	33.71 ± 0.90 32.92 – 35.25	35.01 ± .097 33.40 – 36.00	38.25 ± 1.79 36.80 – 41.25
Eye diameter	13.38 ± 0.35 12.78 – 13.89	12.16 ± 0.37 11.80 –12.66	12.74 ± 0.13 12.63 – 12.92	14.24 ± 0.54 13.54 – 14.73	14.73 ± 0.34 14.34 –15.21

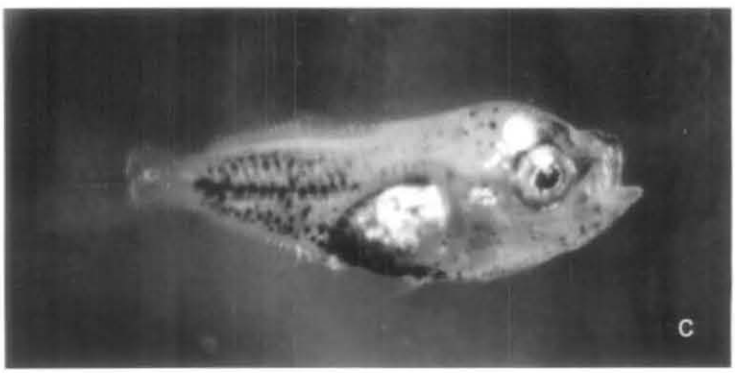
Plate 1. Larval stages of *A. sebae*



Hatchling (1 day old)



Flexion (5 day old)



Post flexion (10 day old)

Early juvenile (15 days)

ii. *Pre flexion larvae* : Caudal rays started appearing by the third day and there was marked change in the head length, and head depth with respect to body length. Maximum mouth opening ranged from 450 to 550 µm.

iii. *Flexion* : Notochord flexion started by the fourth day and was complete by the seventh day. The body became less transparent and there was increased pigmentation. By the seventh day the ratio of total length to standard length increased considerably due to the development of caudal fin. The maximum mouth gape of the larvae ranged from 590 to 660 µm. Head length, head depth and body depth continued to increase. (Plate 1b)

iv. *Post flexion* : By the tenth day when the larvae were examined, they had advanced fin development but had not attained adult coloration. Maximum mouth opening varied from 690 to 730µm. (Plate 1c).

v. *Early juveniles* : Complete fin development and adult colour patterns were noted. Colour started appearing by 11th day. Morphometry of 14 day old juveniles is given in Tables 4 and 5. Maximum mouth opening ranged from 880 to 1026 µm. (Plate 1d).

7.3.2.2. Damselfishes

N. cyanomos and *P. caeruleus*

Morphology and the time taken to attain different stages were identical in both *N. cyanomos* and *P. caeruleus*. So they are described together. The morphometry of *N. cyanomos* larvae is given in Table 6 and ratios with respect to body length in Table 7 and those of *P. caeruleus* are given in Table 8 and Table 9 respectively. The different developmental stages are described below :

i. *Hatchlings* : The hatchlings were slender and transparent. The maximum mouth opening ranged from 147 to 220 µm for *N. cyanomos* and 158 to 189 µm for *P. caeruleus*.

Table 6 . Morphometry of larvae of *N. cyanomos* during different stages of growth.
Mean ± standard deviation in the first line and range in the second line.

Measurements (mm)	Hatchlings	Pre flexion	Flexion	Early juvenile
Total length	1.82 ± 0.21 1.50 – 2.16	4.32 ± 0.25 3.99 – 4.62	5.27 ± 2.22 5.02 – 5.58	18.17 ± 3.14 15.0 – 23.0
Body length	1.72 ± 0.2 1.43 – 2.05	4.16 ± 0.10 3.89 – 4.44	4.86 ± 0.17 4.66 – 5.10	13.17 ± 1.59 12.0 – 16.0
Head length	0.41 ± 0.002 0.37 – 0.44	0.91 ± 0.09 0.81 –1.03	1.20 ± 0.05 1.14 – 1.25	4.58 ± 0.81 4.0 – 6.0
Head depth	0.42 ± 0.03 0.40 – 0.48	0.84 ± 0.10 0.73 – 0.95	1.16 ± 0.02 1.14 – 1.18	3.21 ± 0.24 3.0 – 5.0
Body depth	0.36 ± 0.03 0.33 – 0.40	0.81 ± 0.09 0.73 – 0.92	1.07 ± 0.03 1.03 – 1.10	4.50 ± 0.91 3.50 – 5.50
Eye diameter	0.23 ± 0.02 0.22 – 0.26	0.45 ± 0.03 0.40 – 0.48	0.59 ± 0.02 0.55 – 0.62	1.72 ± 0.34 1.50 – 2.30
Number or specimen	12	5	5	6

Table 7 . Morphometric ratios of larvae of *N. cyanomos* expressed as percentage to body length.
Mean ± standard deviation in the first line and range in the second line.

Measurements (mm)	Hatchlings	Pre flexion	Flexion	Early juvenile
Total length	105.81 ± 1.18 104.43 – 108.52	103.90 ± 1.39 102.54 – 105.76	108.40 ± 1.16 106.92 – 109.70	137.37 ± 8.70 125.0 – 150.0
Head length	23.73 ± 1.52 21.46 – 25.87	21.84 ± 1.26 20.29 – 23.20	24.75 ± 0.36 24.46 – 25.25	34.64 ± 1.69 33.33 – 37.50
Head depth	24.68 ± 2.49 19.80 – 27.97	20.39 ± 1.36 18.76 – 20.72	23.86 ± 0.51 23.14 – 24.46	24.18 ± 1.81 21.88 – 26.92
Body depth	21.25 ± 1.39 19.51 – 23.07	19.64 ± 0.87 18.76 – 20.72	22.02 ± 0.83 20.81 – 22.75	34.02 ± 3.26 29.17 – 39.29
Eye diameter	13.46 ± 0.87 12.50 – 15.38	10.77 ± 0.33 10.28 – 11.09	12.09 ± 0.22 11.80 – 12.37	13.16 ± 1.49 11.54 – 15.63

Table 8 . Morphometry of larvae of *P.caeruleus* during different stages of growth
Mean ± standard deviation in the first line and range in the second line

Measurements (mm)	Hatchlings	Pre flexion	Flexion	Early juvenile
Total length	2.31 ± 0.21 2.02 – 2.59	4.40 ± 0.20 4.18 – 4.69	5.35 ± 0.25 5.06 – 5.72	19.3 ± 2.86 16 – 23.0
Body length	2.19 ± 0.32 1.93 – 2.47	4.21 ± 0.20 3.99 – 4.47	4.88 ± 0.18 4.66 - 5.13	13.9 ± 1.86 12.0 – 16.50
Head length	0.49 ± 0.04 0.44 – 0.51	0.93 ± 0.12 0.81 – 1.06	1.21 ± 0.06 1.14 – 1.28	4.9 ± 0.89 4.0 – 6.0
Head depth	0.47 ± 0.02 0.44 – 0.47	0.89 ± 0.09 0.77 – 0.99	1.17 ± 0.03 1.14 – 1.21	3.20 ± 0.27 3.0 – 3.50
Body depth	0.46 ± 0.02 0.44 – 0.47	0.85 ± 0.10 0.73 – 0.96	1.09 ± 0.05 1.03 – 1.14	4.60 ± 0.96 3.5 – 6.0
Eye diameter	0.23 ± 0.03 0.08 – 0.26	0.46 ± 0.03 0.44 – 0.51	0.59 ± 0.05 0.55 – 0.66	1.90 ± 0.42 1.50 – 2.50
Number or specimen	12	5	5	5

Table 9. Morphometric ratios of larvae of *P. caeruleus* expressed as percentage to body length
Mean ± standard deviation in the first line and range in the second line

Measurements (mm)	Hatchlings	Pre flexion	Flexion	Early juvenile
Total length	105.37 ± 1.04 103.02 – 106.63	104.58 ± 0.80 103.32 – 105.46	109.59 ± 1.14 108.58 – 111.50	138.63 ± 4.23 133.33 – 144.44
Head length	22.33 ± 1.49 19.34 – 24.35	21.91 ± 1.80 20.10 – 24031	24.77 ± 0.34 24.44 – 25.25	35.08 ± 2.26 32.0 – 37.04
Head depth	21.34 ± 1.77 19.03 – 23.98	21.22 ± 1.41 19.55 – 22.71	23.89 ± 0.69 22.81 – 24.46	23.15 ± 1.48 21.21 – 25.00
Body depth	20.85 ± 1.80 18.57 – 23.98	20.22 ± 1.43 18.30 – 22.02	22.44 ± 1.39 20.08 – 23.66	32.84 ± 2.59 29.17 – 36.36
Eye diameter	10.36 ± 0.94 8.91 – 11.40	10.96 ± 0.35 10.43 – 11.41	12.15 ± 0.54 11.63 – 12.87	13.56 ± 1.39 12.00 – 15.15

ii. *Pre flexion* : Initial caudal rays appeared by the tenth day for both the species. There was a slight reduction in the proportion of head length, head depth and body depth for both the species at this stage. The maximum mouth opening ranged from 220 to 290 µm in both the species.

iii. *Flexion* : Flexion started in 12 to 14 days and completed in 18 to 20 days. Morphometry of the larvae at mid flexion (16 days) is given in Table 6 and Table 8 for either species. Stellate melanophores appeared laterally behind the level of anus in the dorsal half. The proportion of the head length, head depth and body length increased. Maximum mouth opening ranged from 440 to 513 µm for *N. cyanomos* and 439 to 549 µm for *P. caeruleus*.

iv. *Early juvenile* : Adult colouration started appearing by the 28th day. Outer rays of caudal fin and middle rays of soft dorsal and anal fin started to elongate in *N. cyanomos*. The ratio of head length and body depth increased and there was no noticeable change in the ratio of head depth for both the species. Maximum mouth gape ranged from 1.5 to 2.0 mm for both the species. The early juvenile of *P. caeruleus* is shown in Plate 2a and that of *N. cyanomos* in Plate 2b.

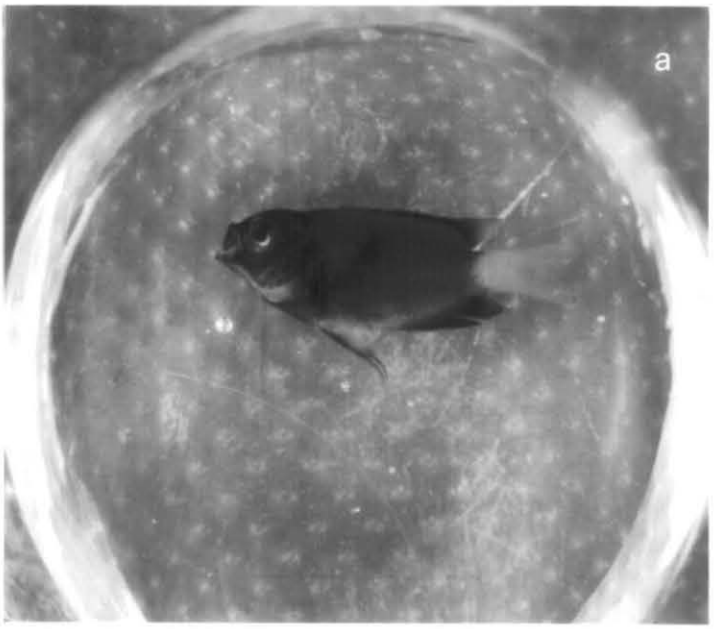
Pomacentrus pavo

The development was slower in *P. pavo* than in the other two species. The morphometric measurements of *P. pavo* during larval stages is given in Table 10 and the ratios to body length in Table 11. The different developmental stages are shown in Plate 3 and described below :

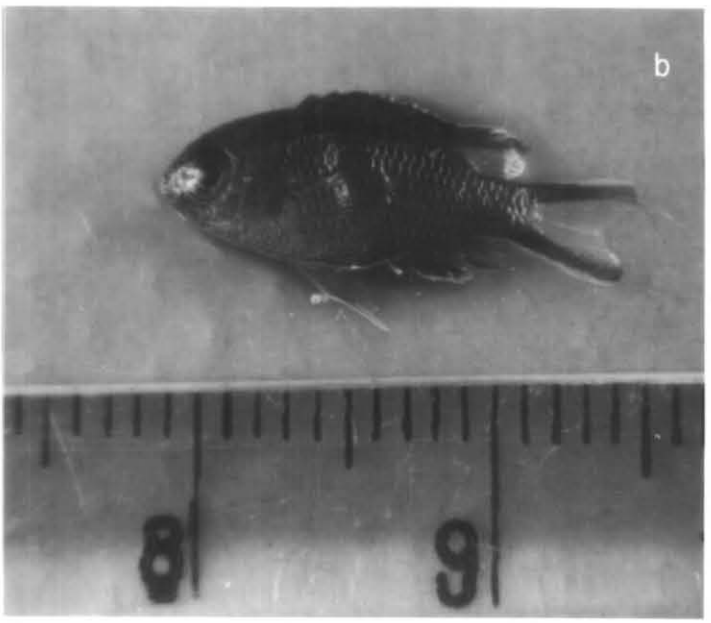
i. *Hatchlings* : The hatchlings were similar to other species of damsel fishes and had a maximum mouth opening in the range of 158 to 221 µm (Plate 3a).

ii. *Pre flexion larvae* : First caudal rays appeared by 12 to 14th day. There was an increase in head length and head depth but the body depth almost remained the same. The maximum mouth opening ranged from 260 to 330 µm. Ten day old larva before the appearance of caudal rays is shown in Plate 3b and a 14 day old larva with initial caudal rays in Plate 3c.

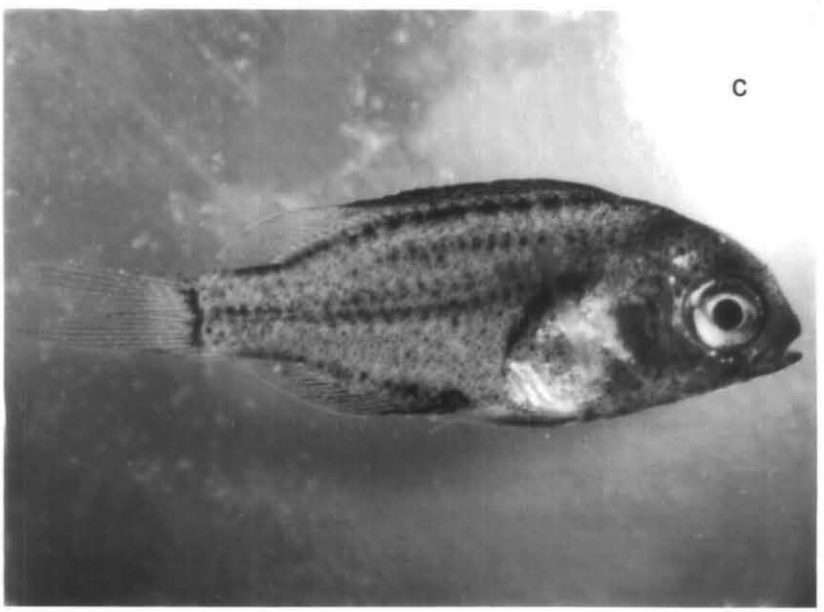
Plate 2. Early juveniles of the damselfishes reared



P. caeruleus



N. cyanomos



P. pavo

Table 10 . Morphometry of larvae of *P.pavo* during different stages of growth

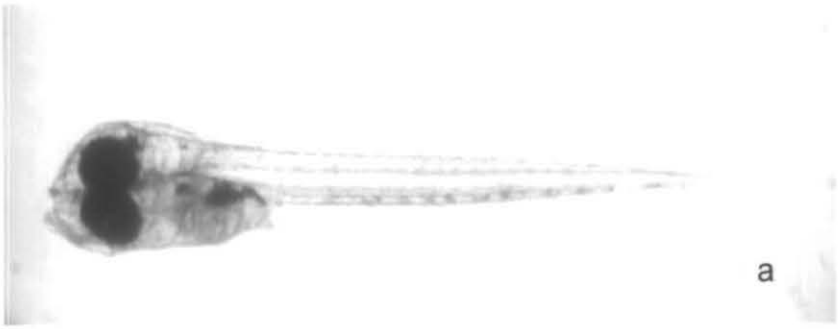
Mean ± standard deviation in the first line and range in the second line

Measurements (mm)	Hatchlings	Pre flexion	Flexion	Early juvenile
Total length	2.08 ± 0.17 1.93 – 2.47	4.61 ± 0.12 4.44 – 4.77	4.85 ± 0.15 4.69 – 5.02	13.58 ± 2.20 11.0 – 16.50
Body length	1.97 ± 0.16 1.83 – 2.31	4.45 ± 0.12 4.33 – 4.62	4.56 ± 0.10 4.44 – 4.66	10.42 ± 1.74 8.50 – 13.00
Head length	0.41 ± 0.03 0.38 – 0.47	0.98 ± 0.05 0.95 – 0.06	1.06 ± 0.06 0.99 – 1.14	3.25 ± 0.61 2.50 – 4.0
Head depth	0.40 ± 0.02 0.38 – 0.44	0.95 ± 0.06 0.88 – 1.03	1.03 ± 0.07 0.95 – 1.10	2.92 ± 0.59 2.0 – 3.50
Body depth	0.39 ± 0.01 0.38 – 0.41	0.88 ± 0.06 0.81 – 0.96	0.95 ± 0.02 0.92 – 0.99	3.25 ± 0.61 2.50 – 4.0
Eye diameter	0.24 ± 0.02 0.22 – 0.25	0.48 ± 0.02 0.44 – 0.51	0.51 ± 0.05 0.48 – 0.55	1.13 ± 0.29 0.80 – 1.50
Number or specimen	12	5	5	6

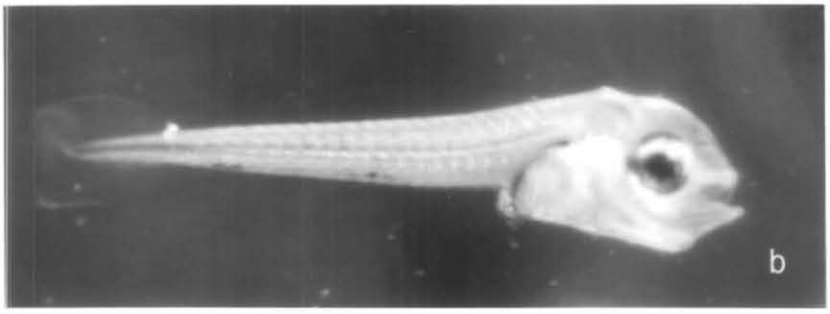
Table 11 . Morphometric ratios of larvae of *P. pavo* expressed as percentage to body length
Mean ± standard deviation in the first line and range in the second line

Measurements (mm)	Hatchlings	Pre flexion	Flexion	Early juvenile
Total length	105.91 ± 0.73 104.66 – 106.99	103.51 ± 0.32 102.54 – 104.36	106.17 ± 1.45 103.96 – 103.73	130.50 ± 2.47 126.92 – 133.33
Head length	20.74 ± 1.04 19.05 – 22.04	22.00 ± 0.57 21.40 – 22.94	23.17 ± 0.87 22.30 – 24.46	31.14 ± 1.74 29.41 – 33.33
Head depth	20.15 ± 1.18 17.75 – 21.67	21.29 ± 1.13 20.32 – 22.71	22.64 ± 1.03 21.40 – 23.61	27.57 ± 2.40 23.53 – 30.00
Body depth	19.85 ± 0.90 17.75 – 20.76	19.62 ± 0.95 18.24 – 20.78	20.86 ± 0.47 20.40 – 21.24	31.14 ± 1.74 29.41 – 33.33
Eye diameter	11.98 ± 0.80 10.82 – 13.44	10.74 ± 0.50 9.91 – 11.09	11.25 ± 0.56 10.55 – 11.80	10.76 ± 1.15 9.41 – 12.50

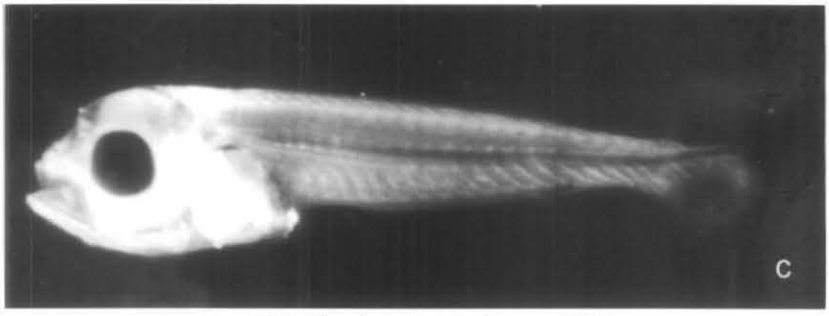
Plate 3. Larval developmental stages of *P. pavo*



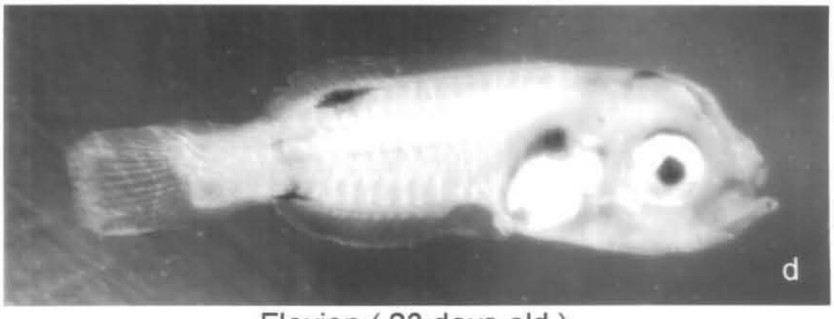
Hatchling (1 day old)



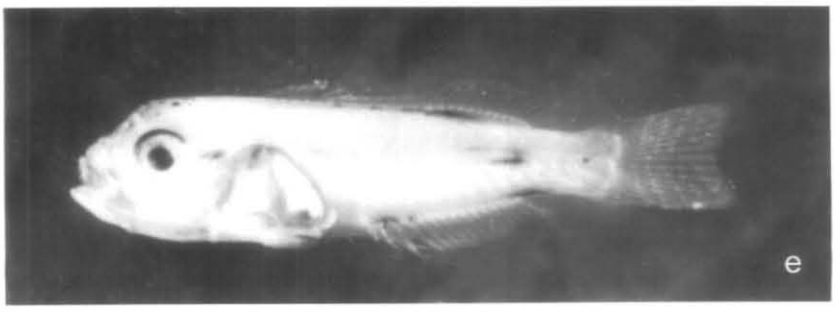
10 day old larva



Pre flexion (14 days old)



Flexion (23 days old)



Post flexion (30 days old)

iii. *Flexion* : Flexion of notochord started by 15 to 18th day and was complete by 23 to 25th day. Description of mid flexion larvae (18 days) is given. Melanophores appeared dorso laterally above the anus and afterwards on the head. Maximum mouth opening was 370 to 440 µm (Plate 3d). Thirty day old larva after completion of flexion is shown in Plate 3e.

iv. *Early juvenile* : Bluish green colour started appearing for the larvae by 37th day. The head length, head depth, body depth and total length increased at this stage. The maximum mouth opening ranged from 1.0 to 2.0 mm (Plate 2c).

7.4. Discussion

Most of the marine fish larvae are very small compared to the larvae of many freshwater fishes. Newly hatched larvae of the groupers (*Epinephelus* spp.) have size less than 2 mm Lim (1993) .The mouth opening of these larvae are too small to accept rotifers. Super small strain rotifers have been used for feeding the larvae in the initial stages. There are reports of successful rearing of groupers and sea bass, *Lates calcarifer* using S S strain rotifers as the first feed to the larvae. Difficulty in identifying and culturing suitable live feed organisms as the first feed is a major constraint in rearing marine ornamental fish larvae also. The damselfish larvae also have comparable sizes to those of the larvae of food fishes and are difficult to rear. Therefore immediate attention must be given for identifying and developing techniques for raising their mass culture.

Green water technique is widely employed in the rearing of marine finfish larvae. This method has been successful for pomacentrid larvae also (Danilowicz and Brown, 1992 ; Job *et al.*, 1997 ; Arvedlund *et al.*, 2000). Green water containing micro algae such as *Chlorella*, *Nanochloropsis* etc. are added to stabilize the water quality by functioning as a nutrient sink (Job *et al.*, 1997).

The green water also reduces light penetration in to the larval rearing tanks. The pomacentrid larvae are very sensitive to light, and in presence of bright light reflections, they exhibit 'head butting syndrome' and consequent mass mortality (Job *et al.*, 1997). Large scale or complete mortality occurred whenever the micro algal density crashed in the larval rearing tanks especially in the first three weeks. The presence of oil film on the water surface can cause lordosis and mass mortality and therefore oil skimmers must be used to reduce this (Lim, 1993). The oil skimmers set up in the tanks increased the survival especially after 10 days of culture.

Clownfish larvae were less sensitive to light than the damsel fishes and were raised in clear water. But they also should be protected from bright light. Arvedlund *et al.* (2000) reported a 16 hour light and 8 hour dark regime for better survival and growth of *Amphiprion melanopus* larvae. A 16 hour lighting was provided in the present study also.

The clownfish larvae accepted rotifers from the next morning after hatching eventhough complete yolk exhaustion occurred after two days. Coughlin (1994) reported that *Amphiprion perideraion* larvae started feeding two days after hatching. Green and McCormick (1999) stated that newly hatched larvae of *Amphiprion melanopus* were capable of accepting external feeds.

The yolk exhaustion in the four damsel fishes occurred by the third day after hatching. They were not found to accept any external food for the first two days eventhough jaws were movable. They were maintained in a medium which was a mixture of zooplankters and ciliates. The larval gut after two days contained ciliates, but it was not clear whether it is a preferred food item for the larvae. Moe (1992) reported that ciliates like *Euplotes* are useful for feeding marine fin fish larvae. But maintaining cultures of ciliates is a difficult process and also little is known about their nutritive value. The possible reason for the

presence of ciliates in the larval gut may be that they were the only particles capable of being ingested by the larvae having mouth gapes less than 200 µm. Eventhough rotifers were present in the rearing medium, they were scanty in the gut of larvae even after 10 days when they had sufficient mouth opening to ingest rotifers. Young (1992) reported the non acceptance of rotifers by certain pomacentrid larvae.

All the species showed a faster growth after the pre flexion stage. The higher growth rate after the initiation of flexion is evident from the growth curve. The growth rate reduced after metamorphosis for anemonefishes. The initial mortality due to transfer of larvae from the broodstock tanks to larval rearing tanks was observed for all the species and in all the trials. The mortality gradually reduced in anemonefishes but unexpected mass mortality occurred in all damselfishes at any time before metamorphosis. The use of oil skimmers increased the survival rate after 10 days of culture.

Alshuth *et al.* (1998) reported dramatic changes in body proportions from pre flexion stage. Similar pattern was observed for *A. sebae* larvae and for other species, and the changes started with notochord flexion. From the start of flexion the ratio of total length to body length increased steadily till metamorphosis due to the development of caudal fin. The proportion of the eye diameter remained almost constant throughout the larval period for all the species.

Different species of anemone fishes have been reared in various parts of the world with good survival rates (Alava and Gomes, 1989 ; Allen, 1991 ; Hoff, 1996 ; Wilkerson, 1998 ; Gopakumar *et al.*, 1999 ; Ignatius *et al.*, 2001). But attempts for rearing damsel fishes which produce larvae of less than 2.5 mm in length had only limited success. Eventhough there are reports of rearing of different damselfishes, the survival rates were mostly low (Danilowicz

and Brown, 1992). In the current study also, eventhough experimental success has been achieved in the rearing of four damsel fish species the survival has been low. It is felt that higher and consistent survival rates can be achieved with focussed research on larval rearing systems, feeding strategies and water quality parameters.

SUMMARY

Marine aquarium keeping is rapidly gaining popularity among aquarists and there is an increasing demand for marine ornamentals in recent years. As a result, the exploitation of the coral seas rapidly increased because most of the most of the marine ornamental animals are inhabitants of the coral seas. Such indiscriminate exploitation of coral reef areas will result in the destruction of corals which are the resultant of hundreds of years of reef building activity of the coral forming organisms. The destruction of the corals will in turn lead to the depletion of the rich biota associated with it. Therefore hatchery technology of important ornamental fishes has to be developed to meet their increasing demand. Pomacentrids are one of the most important groups of fishes suitable for marine aquaria which include the clownfishes and damselfishes. Hence, research work on their availability and abundance, reproductive biology, breeding patterns, behaviour and larval rearing methods which can yield valuable information for developing technologies for their hatchery production have to be undertaken on a priority basis.

A study of the systematics of commonly available pomacentrids was conducted mainly off Vizhinjam on the south west coast of India. A total of 24 species belonging to 9 genera were collected of which 18 species were available along Vizhinjam coast. The species which were collected from Vizhinjam are *Abudefduf bengalensis, A. notatus, A. septemfasciatus, A.sordidus, A. vaigiensis, Chrtsiptera biocellata, C. unimaculata, Neopomacentrus cyanomos, N.nemurus, N. sindensis, N. taeniurus, Neoglyphydodon bonang, Plectroglyphydodon lacrymatus, Plectroglyphydodon leucozonus, Pomacentrus. caeruleus, P. pavo, P. adelus and P. proteus. Amphiprion sebae* was collected from Rameswaram, *Chromis viridis, Chrysiptera rollandi, Dascyllus aruanus, D. carneus, D. trimaculatus* and *P.*

pavo were collected from Minicoy. Among these *Chrysiptera rollandi, Neopomacentrus sindensis, Neoglyphydodon bonang, Plectroglyphydodon leucozonus, Pomacentrus adelus* and *P. proteus* have been reported as new records from Indian waters. Notes on the colour variations of the above species were also made.

One of the most important aspects of the reproductive biology of pomacentrids is the phenomenon of sex change exhibited by many members of the family. Reproductive biological aspects of two species *viz., Amphiprion sebae* and *Neopomacentrus cyanomos* were investigated in the present study. Size frequency distribution of males and females clearly indicated protandry in the anemonefish and protogyny in the damselfish. In *A. sebae* the size of females ranged from 65 to 95 mm standard length and all the individuals above 85 mm were females. The smaller size group upto 65 mm were all males and both males and females were present in the length range 65 to 85 mm. Male to female ratio was 5 : 1 in the entire sample and 1.3 : 1 in the overlapping range. In *N. cyanomos* the smaller size group upto 65 mm standard length were all females and all the individuals above 80 mm were males. The female to male ratio in the entire sample was 4.6 : 1 and in the length range of 65 to 80 mm the ratio was 0.83 : 1.

Histological analysis of gonads confirmed the ambisexual nature of the anemonefish testis. Gonads in the transitional stage contained lesser testicular area than ovarian areas. Ovaries with mature eggs did not contain any testicular tissue. This points to the possible irreversible nature of the sex change. However, ambisexual gonads were not obtained from *N. cyanomos*. So the sex transformation in *N. cyanomos* may be slower than that of the anemonefish.

The ovaries of *N. cyanomos* were categorized into three maturity stages *viz.*, immature, maturing and ripening according to the size and development of intra ovarian eggs. In the anemonefish a fully transformed gonad resembled a maturing ovary which may swiftly become a ripening ovary. The ripening ovary of both species contained mainly 3 different size groups of eggs and thus the egg development is mostly a continuous process enabling them to spawn throughout the year.

In pomacentrids, sexual maturity and spawning are socially controlled. The anemonefish was monogamous and all other damselfishes studied were polygynous at least occasionally under captive conditions. Breeding group formations from juvenile populations were studied for *A. sebae* and *N. cyanomos*. In the anemonefish two juveniles grew rapidly than other individuals and became the functional pair, the largest one being the female. In case of *N. cyanomos* one fish grew faster than the other and became the functional male. All other fishes in the group were functional females. Spawning started after 12 months in *A. sebae* and after 8 months in *N. cyanomos*.

Experiments were done using the above two species to observe whether two individuals of the initial sex that were kept together in a container were able to form a functional pair after transformation in one of them. Pairing and transformation was observed in anemonefish in all the three trials. However, in the case of *N. cyanomos*, both fishes in each group were functional or maturing females. Therefore more number of fishes of the initial sex may be required to cause a female to male transformation in one of them. From this, it is inferred that polygyny is favoured in the damselfish and monogamy in the anemonefish.

The possibility of inducing a sex change in anemonefish was studied by removal experiments. Functional females were removed from breeding pairs and subadults smaller than the functional males were introduced with them. Male to female transformation occurred in the functional males in all the groups. The duration between the introduction of the subadult and first spawning after transformation varied between 61 to 135 days.

Broodstock of seven species were developed and observations were made on their captive breeding patterns. All the five species - *A. sebae, N. cyanomos, P. caeruleus, P. pavo* and *D. carneus* - for which round the year data were collected exhibited continuous spawning without any seasonality. *N. nemurus* and *N. sindensis* also spawned continuously during the six month period of observation. None of the species showed any lunar periodicity of spawning. The spawning behaviour of all the fishes were similar to the general pattern described earlier.

The average number of eggs laid in a single spawning by the anemonefish was 569 ± 181.3 and ranged from 100 to 1450. The average annual fecundity of a pair was 10231 ± 1473 and the number of spawnings per year by a pair was 18.3 ± 2.01. The spawning cycle was mostly of 11 days for *A. sebae* and 12 days for *D. carneus*. All other species spawned continuously and a clutch often contained eggs laid on different days. Average clutch size was 4065 ± 421.62 for *D. carneus*, 2656 ± 78.74 for *P. pavo*, 2867 ± 137.21 for *P. caeruleus*, 3611 ± 203.11 for *N. cyanomos*, 2788 ± 282. 24 for *N. nemurus* and 4912 ± 276. 74 for *N. sindensis*. In *P. pavo* the new eggs were mostly laid contiguous to the existing clutch if the latter is one or two days old but laid as a separate clutch afterwards. Parental care was exhibited exclusively by male in all damselfish species whereas females also took part occasionally in clownfish.

The early embryological development was studied for all species which spawned in captivity. The egg size and development were similar for the five species – *Neopomacentrus cyanomos, N. nemurus, N. sindensis, Pomacentrus caeruleus* and *P. pavo.* The eggs were capsule shaped in all the above species and also in the anemone fish. In *D. carneus* the size of eggs were less and also it was oval in shape. All the species laid demersal attaching type eggs typical to the family. The incubation period was mostly 7.5 to 8.5 days for anemonefish, 2.5 days for *D. carneus,* and 3.5 days for the rest of the species studied.

Larvae were reared successfully for the five species : *Amphiprion sebae, Neopomacentrus cyanomos, N. nemurus, Pomacentrus caeruleus* and *P, pavo.* The newly hatched larvae of all these species were pelagic with a single finfold. Complete yolk exhaustion occurred in 2 to 3 days for all the species studied. The newly hatched larvae of *A. sebae* measured 3.97 ± 0.19 mm, *N. cyanomos* 1.82 ± 0.21, *P. caeruleus* 2.31 ± 0.21 and *P. pavo* 2.08 ± 0.17 mm. Greenwater system was used for rearing damselfish larvae while a flow through system was used for rearing the anemonefish larvae. The anemonefish larvae were fed with rotifers initially and later with *Artemia* nauplii and *Moina.* The metamorphosis and appearance of adult colour pattern occurred in 11 to 14 days. The percentage survival till metamorphosis ranged from 6.6 % to 74 % with a mean of 35.4 % in 12 experimental trials with anemonefish larvae. The damsel fish larvae were found to ingest ciliates present in the microalgal debris for initial few days. Later they were fed with boiled and finely smashed mussel ovaries and tissues filtered through bolting silk of appropriate mesh size. As they grew they were fed with freshly hatched *Artemia* nauplii and *Moina* till metamorphosis. The larval duration was longer for all the damselfishes. It ranged from 28 to 33 days for *N. cyanomos, N.nemurus* and *P. caeruleus* and 37 to 41 days for *P. pavo.* The development of the larvae till metamorphosis was also studied for four species.

The studies on reproductive biology and captive breeding have to be intensified to generate information required for the hatchery production of pomacentrids. Observations from the present study indicated that pomacentrids are a group of fishes of ornamental importance which can be maintained and bred in captivity rather easily but the larval rearing of many species other than anemonefishes is the most complicated aspect. Therefore, more thrust must be given to develop technologies of mass culture of appropriate live feeds and suitable larval rearing systems which can pave the way for the development of ornamental fish mariculture capable of meeting the increasing demand.

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